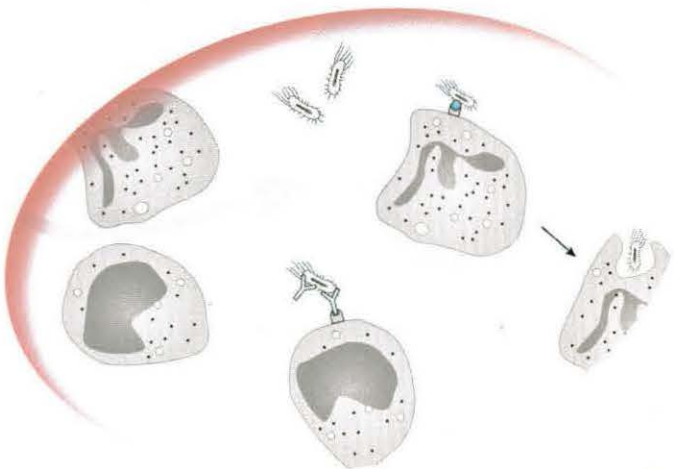
QUICK
LOOK
MEDICINE

USMLE STEP 1
A VISUALLY ORIENTED REVIEW

Immunology

MARK J. MAMULA, PH.D.



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Preface

Quick Look Medicine: Immunology is designed to provide a concise overview of the key elements of immune responses. It is clear that the immune system evolved to protect the host from infectious microorganisms. In protecting the host, the end product of immune responses incorporates a vast array of interactive cells, cell surface receptors, soluble macromolecules, and tissues. Our understanding of immune responses is far from complete, although the past decade has provided a wealth of new information in the areas of cytokines and chemokines, the development of B and T lymphocytes, and host-pathogen interactions. It has been our attempt to compartmentalize important features of immune responses and present them in a manner that resembles the normal development of immunity.

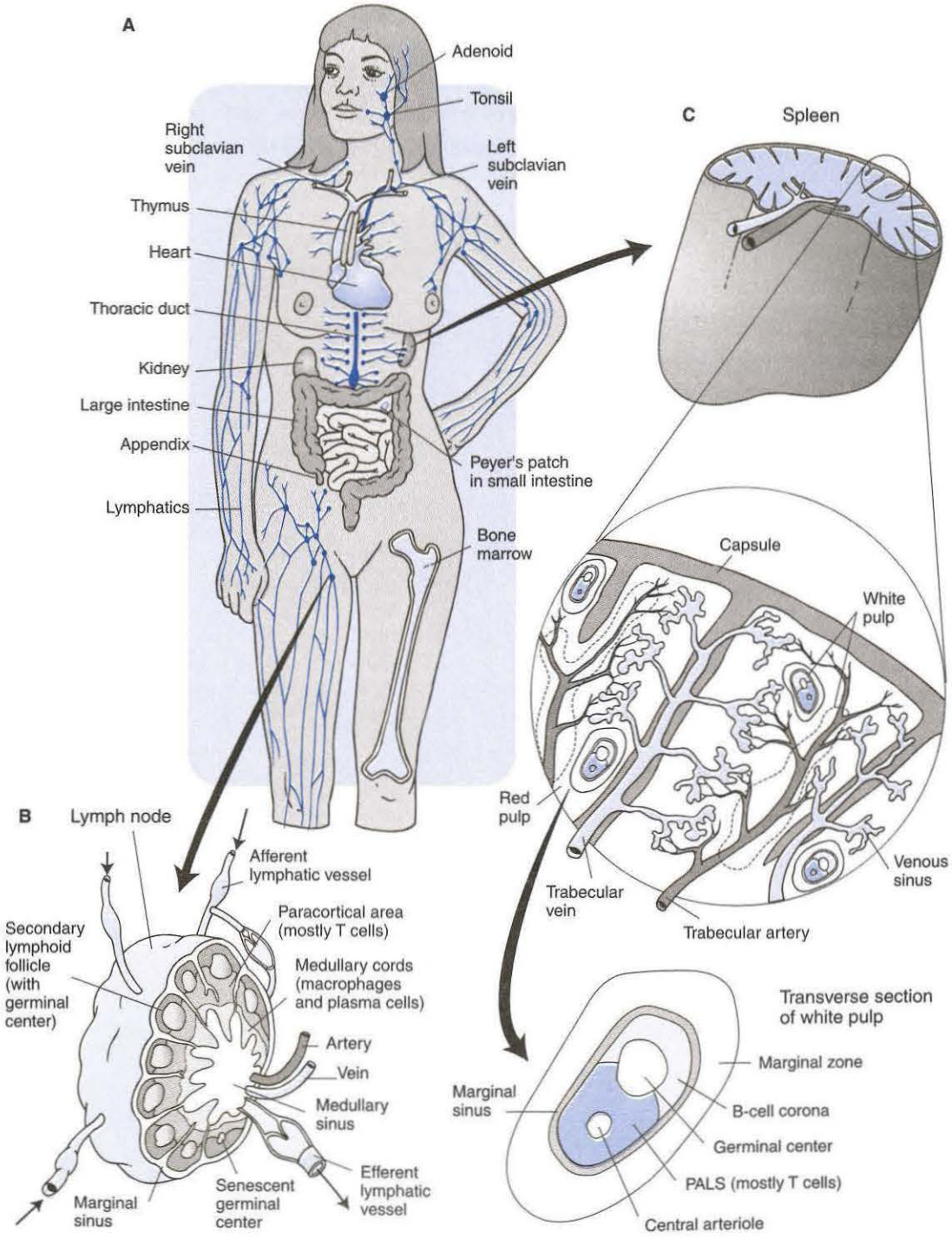
This test is intended to jog the memory of students with a rudimentary understanding of the immune system and provide the latest information in areas such as lymphocyte development, AIDS, and other clinically relevant immunologic syndromes. In earlier chapters we provide a general description of the components of the immune responses followed by chapters that cover individual topics in greater depth. The final chapters integrate basic immunology with applied clinical topics including immune-deficiency and autoimmune syndromes, concepts of vaccination, and laboratory analysis of immune responses. Pay particular attention to the key terms that are found in italics throughout the text. While we have avoided the use of immunologic jargon in most areas, some terms are essential to the language of immunology. A glossary of important terms is provided at the end of the textbook.

Most immunology textbooks provide experimental evidence in support of concepts that have emerged in this field. Historically, the field is founded in well-defined laboratory investigations. Advances in areas such as transplantation, the major histocompatibility complex, and immunologic tolerance have risen from animal models and investigations at the laboratory bench. The highly focused nature of this text does not allow a detailed description of experimental basis of immunology concepts and the reader is encouraged to supplement his or her interests with other basic immunology textbooks and original research articles. The illustrations and tables have been designed to incorporate the significant points of each chapter and the questions should test your overall understanding of the concepts. We encourage your comments and criticism of this textbook, which can be communicated through our website at *www.fencecreek.com.*

Mark J. Mamula, Ph.D.

I would like to dedicate this textbook to my parents, Fran and Emil Mamula, who have nurtured my pursuits in life, whether it be in science or in sport, and have provided the motivation for excellence in whatever endeavors I have chosen. I an indebted to friend and colleague, Dr. Charlie Janeway, who is partly to blame for my genuine interests in immunology since his ethusiasm for this field infects all of those who have worked with him. I would also like to thank Ms. Patricia Melillo for her magnificent help at th computer keyboard in generating this work. To my children, Lindsay, Sarah and Katie who willingly follow me to the laboratory and ask simple yet profound questions such as "how does that work, Dad?". Finally to my wife Ann, whose unconditional love and support permeates my life.

1 Major Components of the Immune System



The immune system represents a collection of central and peripheral organs, cellular components, and soluble macromolecules that collaborate in protecting the body from foreign infectious agents, such as bacteria and viruses as well as tumor cells. The *central* or *"primary" lymphoid organs* of the immune system, the *thymus* and *bone marrow*, are responsible for the early development of lymphoreticular cells, or leukocytes, originating from pluripotent bone marrow stem cells. The neonatal liver is also a primary lymphoid organ where lymphoid cells originate, although this function of the neonatal liver ceases after birth.

Two major cell lineages develop in the bone marrow: lymphoid cells and myeloid cells. Lymphoid cells eventually continue development into either B or T lymphocytes while myeloid cells develop into a variety of end-stage immune cells including neutrophils, basophils, eosinophils, macrophages, platelets, and erythrocytes.

Additional development of immune cells occurs in *secondary lymphoid organs*: the *lymph nodes*, *spleen*, *gut-associated lymphoid organs* (GALT), and *mucosal-associated lymphoid organs*, collectively termed *MALT*. Secondary lymphoid tissues also serve to trap circulating antigens for presentation to lymphoid cells. The development of immune responses with specificity to individual antigens occurs primarily in these peripheral tissues.

Bone Marrow

All cells of the immune system develop from a single type of undifferentiated progenitor cell in the bone marrow. Maturation of erythrocytes, platelets, monocytes, and B lymphocytes occurs in the bone marrow. In this way, the bone marrow can also be considered a secondary lymphoid organ. Precursor cells of T lymphocytes, natural killer (NK) cells, dendritic cells, and mast cells emigrate from the bone marrow to continue development in peripheral tissues.

Thymus

The thymus is an organ that develops from folds of the third and fourth mesodermal pharyngeal pouches during embryogenesis, accounting for its anatomic location in the mediastinum. The thymus reaches maximal size by the time of puberty and slowly involutes throughout life, diminishing in capacity for lymphocyte development. T-cell development, so-called positive and negative selection, is the principal function of the thymus (*t*hymus = *T* cell). However, more than 90% of progenitor bone marrow cells that enter the thymus for development die by a mechanism termed *programmed cell death*, or *apoptosis*. Thymic cell death is a mechanism thought to be responsible for clearing the T-cell repertoire of autoreactive T cells. T cells that survive thymic selection enter the circulation to populate secondary lymphoid organs, and await activation by their specific antigens.

The thymus is rich in connective tissue and is divided into two main substructures, the cortex and medulla. The thymic cortex is the site of rapid cell division that is marked by dense populations (*dark zone*) of thymocytes surrounded by epithelial cells and macrophages. The medulla (*light zone*) is less densely packed with lymphocytes, reticular cells, macrophages, and neutrophils.

The congenital condition DiGeorge syndrome is an absence of thymic tissue resulting in no T-cell immunity and poorly developed B-cell immunity. Patients with DiGeorge syndrome often succumb to severe and recurrent infections.

Lymph Nodes

Lymph nodes are the principal secondary immune tissues that generate B- and T-cell immune responses to most infectious agents. As such, they constitute sites that are important in the adaptive immune responses. Peripheral lymphocytes continuously circulate through lymph nodes via the efferent lymphatics. Plasma that is filtered through the capillary beds feeds the lymph nodes with peripheral lymphocytes. Lymphatic fluid enters the lymph node via the efferent vessels and leaves by afferent vessels through the thoracic duct and finally back to the blood via the left subclavian vein. Lymph nodes may enlarge as much as five times their original size when they are handling an infection. Lymph node swelling is due to the proliferation of antigen-specific B and T lymphocytes that are called to fend off an infectious agent.

The lymph node architecture is highly compartmentalized with specific sites for B- and T-lymphocyte exposure to antigenic stimulation. The outer capsule, or marginal sinus, is composed of sinuous connective tissue. The cortex exists just below the capsule and consists of discrete lobules with germinal centers rich in B lymphocytes that are perfused by lymph from the subcapsular and peritrabecular sinuses. Germinal centers are sites of B-cell activation by antigens causing B-cell proliferation and development into antibody-secreting plasma cells.

Just below this region is the paracortex, a site where T cells are activated by antigen presented by macrophages and dendritic cells. Activated B cells, plasma cells, and T cells eventually migrate into the medullary sinus and out through the efferent lymphatics.

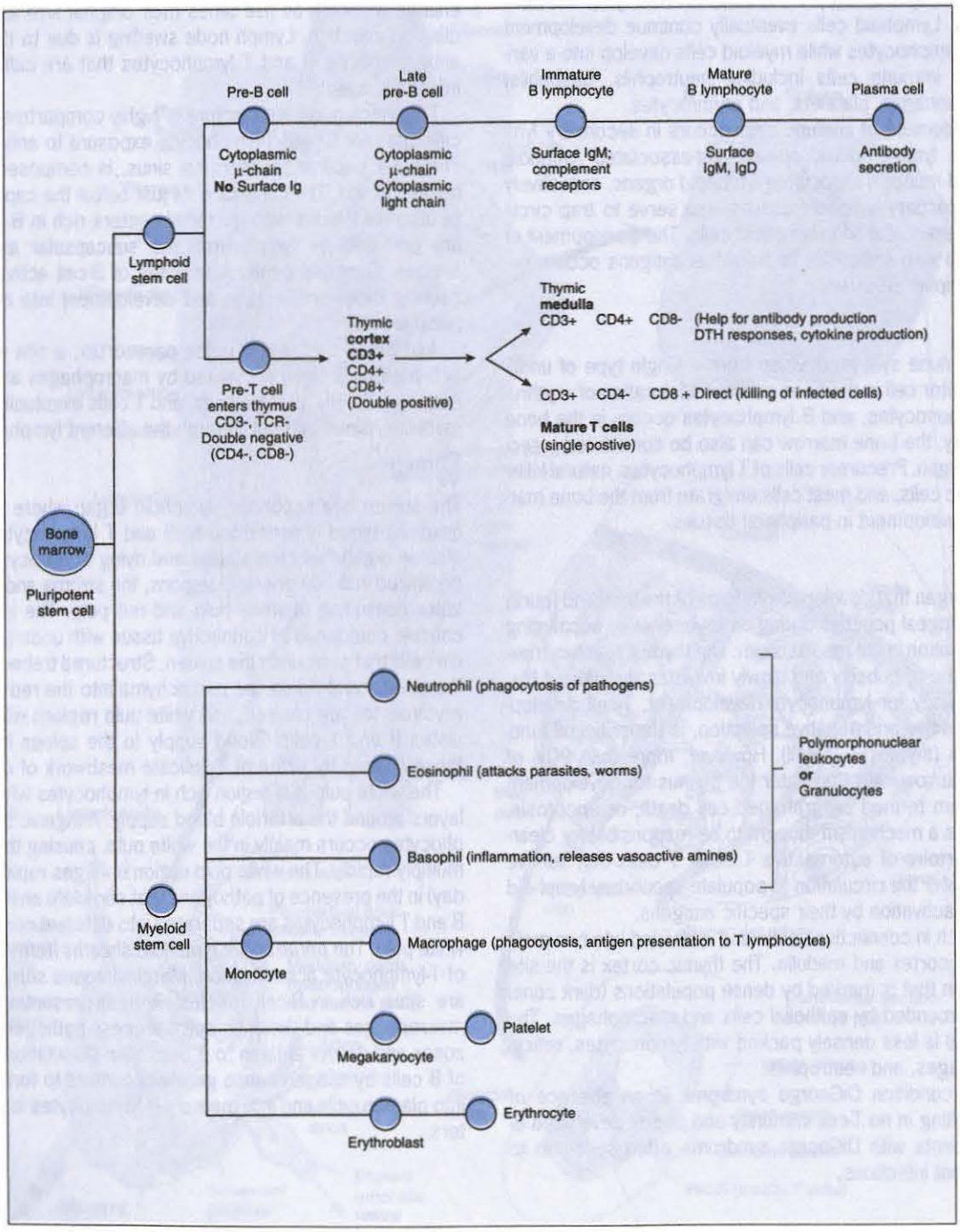
Spleen

The spleen is a secondary lymphoid organ where antigen collected from the blood is presented to B and T lymphocytes. The spleen is also an organ that clears aged and dying erythrocytes. The spleen is organized into two principal regions, the stroma and parenchyma, the latter consisting of white pulp and red pulp. The stroma is a dense capsule composed of connective tissue with underlying smooth muscle cells that surrounds the spleen. Structured trabeculae branch from the stroma and divide the parenchyma into the red pulp where dying erythrocytes are cleared, and white pulp regions where antigen stimulates B and T cells. Blood supply to the spleen is filtered through these regions by virtue of a delicate meshwork of connective tissue.

The white pulp is a region rich in lymphocytes where tissues are in layers around the arteriole blood supply. Antigenic stimulation of lymphocytes occurs mainly in the white pulp, causing the lymphocytes to multiply rapidly. The white pulp region enlarges rapidly (within a single day) in the presence of pathogens that stimulate an immune response. B and T lymphocytes are separated into different compartments in the white pulp. The *periarticular lymphoid sheaths* (termed *PALS*) are sites of T-lymphocyte accumulation. Marginal zones surrounding the PALS are sites rich in B-cell follicles. Antigen-presenting cells, primarily macrophages and dendritic cells, process pathogens at the marginal zones and deliver antigen to B cells. The stimulation and proliferation of B cells by antigen cause *germinal centers* to form. B cells mature into plasma cells and into memory B lymphocytes in the germinal centers.

For more information see Anderson, *IMS: Immunology*. Fence Creek, chapter 3.

2 Cells of the Reticuloendothelial System



All cells of the immune system, or reticuloendothelial system, originate from pluripotent bone marrow stem cells. Once leaving the bone marrow, stem cells relocate to various tissues where they have the capacity to develop into all of the leukocytes important in immune responses. For example, bone marrow cells that locate to the thymus develop into T lymphocytes. Cells that migrate to both fetal liver and other lymphoid organs (such as the bursa of Fabricius in birds) develop into B lymphocytes. In adults, B-lymphocyte development can occur in the bone marrow itself. Lymphoid-specific cells develop based on the exposure to soluble growth factors and particular signaling molecules on the surfaces of other cells such as endothelial cells and antigen-presenting cells (APCs). Other growth stimuli include compounds such as erythropoietin, thymosin, granulocyte-macrophage colony-stimulating factor (GM-CSF), as well as self-antigen and foreign antigen.

Bone marrow cells develop initially into one of two fundamental stem cell lineages, termed *lymphoid* and *myeloid*. Lymphoid stem cells differentiate into B-cell and T-cell subsets while myeloid stem cells develop into macrophages, granulocytes (neutrophils, basophils, and eosinophils), platelets, and red blood cells.

B lymphocytes are the primary cells that synthesize and secrete immunoglobulin (Ig). The end-stage B cell that secretes antibody is termed *plasma cell*. B lymphocytes can also be effective APCs in the stimulation of T-cell responses. This APC function is mediated by the uptake of foreign antigen into the B cell followed by its processing and presentation on the surface with major histocompatibility complex (MHC) class I or class II protein. B lymphocytes undergo a series of well-defined developmental stages on their way to becoming plasma cells. These stages are characterized by the specific proteins expressed on their surface (**Figure**). Early B-cell development occurs in the fetal liver and continues after birth in the bone marrow. B-cell development in the bone marrow depends on adhesion interactions with *stromal cells*, which are nonlymphoid cells that secrete growth factors (stem cell factor and interleukin-7) that promote pre-B-cell development. As pre-B cells begin to express surface Ig, interaction with self-proteins may signal autoreactive B cells to die by *apoptosis* (*programmed cell death*) or become *anergic* (unresponsive). B cells specific for foreign antigen migrate out of the bone marrow and into peripheral lymphoid organs, where they await stimulation by foreign antigen.

Bone marrow stem cells that migrate to the *thymus* differentiate into subsets of *T lymphocytes*. Cell subsets are defined by surface proteins expressed on mature T cells, either *CD4* or *CD8*. As described in detail in later chapters, CD4+ T cells can provide help to B cells for antibody synthesis, participate in delayed-type hypersensitivity (DTH), and directly kill infected cells, the latter a function of CD8+ T cells (cytotoxic T cells).

T-cell development undergoes a series of carefully programmed stages leading to the release of mature T cells. T cells first enter the thymus as *double-negative* (CD4−, CD8−) cells having no T-cell receptor (TCR). Thereafter, low levels of TCR and both CD4 and CD8 become expressed on the surface (termed *double positive*; CD4+, CD8+). A process known as *positive selection* in the thymus signals pre-T cells to develop and to lose either the CD4 surface protein or the CD8 protein. This step is followed by the process of *negative selection* where T cells encounter self-antigen and are signaled to die. Negative selection occurs by the interaction of the TCR on early developing T cells with self-antigen bound to MHC class I or class II molecules on thymic APCs (epithelial cells or macrophages). Negative selection functions to deplete the T-cell repertoire of self-reactive (autoimmune) cells. Approximately 2% of all bone marrow cells entering the thymus survive positive and negative selection and are released into the peripheral circulation.

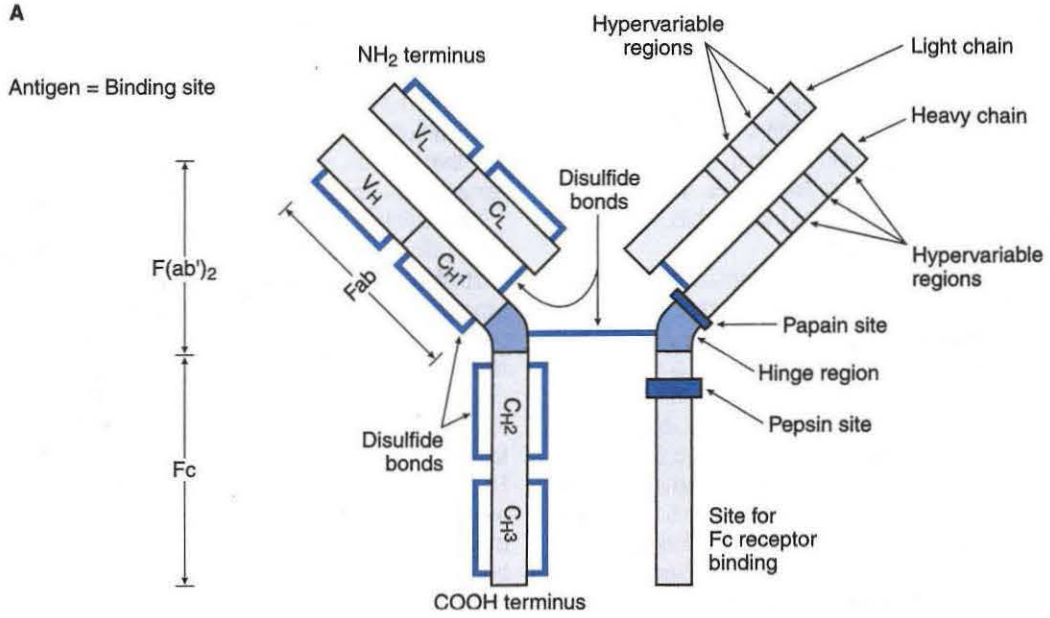
The *myeloid lineage* develops into three types of *granulocytes*, also called *polymorphonuclear leukocytes (PMNs)* because of their multilobed nuclear morphology. *Neutrophils* are the most abundant granulocyte in the immune system and are important cells for the phagocytosis of foreign antigens such as bacteria. Neutrophils are short-lived cells bearing receptors for the terminal region of Ig molecules (Fc receptors) that aid in their phagocytosis of antibody-coated (opsonized) bacteria. A second type of granulocyte, *eosinophils*, contains large granules filled with compounds toxic to infectious agents. Eosinophils are thought to be important in the killing of parasites such as worms. Finally, *basophils* are important granulocytes in the inflammatory response of allergy (type I hypersensitivity reactions). IgE bound to the surface of basophils triggers the release of vasoactive amines and heparin from intracellular granules.

Myeloid stem cells also develop into *monocytes* in the circulation and later become *macrophages* after taking up residence in peripheral tissues. Macrophages are another important phagocytic cell in destroying antibody-coated bacteria. Macrophages also are a major APC in stimulating T-lymphocyte responses and are able to synthesize certain cytokines and complement proteins. Significant numbers of macrophages can be found in the lung (alveolar macrophages), liver (known as Kupfer cells), and peripheral lymphoid organs.

Platelets also originate via the myeloid lineage and are responsible for blood clotting events and in the release of some inflammatory cytokines. Finally, *erythrocytes*, or red blood cells also arise by the stimulation of myeloid stem cells with growth factors such as erythropoietin.

3A Antibodies

A



B

	Immunoglobulin								
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Molecular weight (kda)	146	146	165	146	970	160	160	184	188
Serum concentration (mg/ml)	9	3	1	0.5	1.5	3.0	0.5	0.03	$5x10^{-5}$
Half-life in serum (days)	21	20	7	21	10	6	6	3	2
J chain	–	–	–	–	+	+	+	–	–
Classical pathway complement activation	++	+	+++	–	+++	–	–	–	–
Opsonization	+++	–	++	+	–	+	+	–	–
Placental transfer	+++	+++	+++	+++	–	–	–	–	–
Binding to macrophages and phagocytes	+	–	+	–	–	–	–	–	+
Sensitization/binding to mast cells and basophils	–	–	–	–	–	–	–	–	+++
Antiviral activity	+	+	+	+	–	+	+	–	–
Bacterial toxin neutralization	++	++	++	++	+	++	++	–	–

Immunoglobulin (Ig), or antibody, is the product of the adaptive B-lymphocyte response to foreign antigen or pathogens. Each Ig is characterized by its specificity, isotype (class), and affinity for the antigen to which it binds. Antibody molecules have two distinct functions based on the biologic activities at either end of the molecule. The variable region, termed $F(ab')_2$ (*F*ragment, *a*ntigen *b*inding) at the amino terminus end binds specifically to antigen via several noncovalent interactions. The hinge region at the base of the $F(ab')_2$ allows important flexibility to the Ig structure. In contrast, the carboxy terminus end of the molecule is known as the Fc (*c* = crystalline) region and has a role for clearing immune complexes by binding to cell surface Fc receptors. The Fc site is where the first complement component, C1q, binds and initiates the complement cascade. As described earlier, B lymphocytes only secrete antibody after stimulation of membrane-anchored Ig molecules by their specific antigen.

Antibody Structure

All antibodies have a similar basic structure of 4 polypeptide chains (**Part A**). One polypeptide, of approximately 50 kd, is known as the *heavy chain* while a smaller polypeptide, of approximately 25 kd, is termed the *light chain*. All antibody monomers are composed of 2 heavy chains and 2 light chains. Heavy chains are linked together by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. Five basic Ig classes, termed *isotypes*, exist based on the type of heavy chain present in the Ig. Each antibody isotype is unique regarding timing and location of secretion, the capacity to bind to cell surfaces, the ability to activate the complement cascade, and the ability to mediate inflammation. These varied activities evolved to aid in defense against the many pathogens encountered in a lifetime. Historically, antibody structure was examined by enzyme digestion; *pepsin* cleavage produces a $F(ab')_2$ fragment and free heavy-chain Fc peptides. *Papain* digestion produces 2 monovalent Fab fragments and the intact Fc fragment (two peptides joined by disulfide bonds).

Immunoglobulin G

IgG, the major gammaglobulin in human serum (approximately 12 mg/ml), is the major isotype produced upon secondary antigenic stimulation. IgG is a monomer with 2 heavy and 2 light chains with a total weight of 150 kd. Four subclasses of IgG (designated IgG1, IgG2, IgG3, and IgG4) are found in human serum. The half-life of serum IgG ranges from 7 to 21 days depending on the subclass. IgG is the only isotype able to cross the placenta and provide protection to the newborn during the first several months of life. IgG is the major Ig subclass responsible for opsonization, with a key role in clearing bacteria by immune complex formation. Finally, IgG participates in complement activation and the subsequent lysis of target antigens. All IgG subclasses bind complement, with the exception of IgG4.

Immunoglobulin A

IgA is present in human serum (approximately 3 mg/ml) and exists primarily as a monomer with lesser amounts found as dimers. As with IgM, dimers of IgA are linked by disulfide bonds and J (joining) polypeptide chains. IgA is also found in other extracellular secretions such as saliva, tears, colostrum, and urinary tract and intestinal tract secretions. Secretory IgA (sIgA) is a first line of defense against respiratory and GI tract pathogens since it is excreted across epithelial cells and into the mucus. sIgA protects by binding adhesion molecules on bacterial surfaces, thereby preventing their attachment and colonization within these tissues. IgA also opsonizes foreign antigen and facilitates clearance by binding to membrane Fc receptors on neutrophils.

Immunoglobulin M

The structure of IgM is unique. IgM consists of 5 monomers linked together by disulfide bonds located in the Fc regions as well as by J chains. Its pentameric structure makes it the most effective Ig isotype to activate the complement cascade. Cross-linked immune complexes activate complement most efficiently. Upon immune response to foreign antigen, IgM arises earliest among all Ig isotypes. Membrane-anchored IgM also serves as an antigen receptor on the surface of B lymphocytes. Upon antigenic stimulation, B cells then transport and insert IgD antibody into the cell surface as a receptor. Although phagocytic cells do not possess Fc receptors for IgM isotypes, IgM contributes to the opsonization and clearance of pathogens by virtue of its enhanced ability to bind complement. Complement-bound immune complexes are engulfed and destroyed via complement receptors on phagocytic cells.

Secretory forms of IgM are also produced in local tissues; this isotype is the principal antibody synthesized by the fetus. The serum half-life of IgM is short (approximately 10 days). The J chain of Ig is composed of a 15-kd polypeptide chain that joins the Fc regions of IgM. The pentameric structure of IgM enhances the binding strength of this Ig for each antigen, as 10 antigen-binding sites exist on the pentameric IgM molecule. IgM antibodies often bind antigens with repeating determinants (epitopes) such as polysaccharides, which are components of bacterial cell walls. Due to its large molecular size, IgM does not cross the placental barrier.

Immunoglobulin D

With the exception of IgE, IgD is found in the lowest serum concentration among all Ig isotypes. IgD has a monomeric structure whose function is primarily as a surface receptor on B cells. Antigen binding to membrane IgD stimulates B lymphocytes to synthesize and secrete antibodies of other isotypes. Serum IgD is extremely labile, having a half-life of only 3 days.

As receptors for antigen, both IgM and IgD are expressed simultaneously on the B-cell surface. These Ig molecules extend approximately 25 amino acids of the heavy-chain Fc region into the hydrophobic membrane of the B cell. The membrane domain of IgM or IgD is absent in the secreted form of Ig. The cytoplasmic tail of membrane Ig is associated with specialized signaling molecules, tyrosine kinases, which initiate activation of the B cell.

Immunoglobulin E

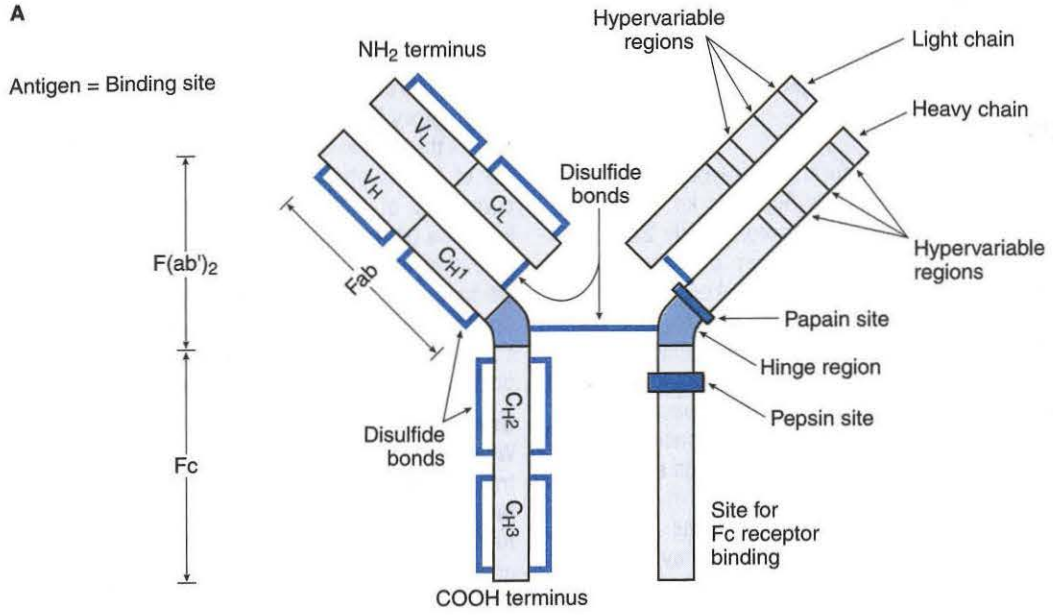
Serum IgE has the lowest serum concentration and shortest half-life (2 days) of all Ig isotypes. IgE exists as a high-molecular-size monomer with an extra domain not found in other isotypes (such as IgG or IgA). IgE is secreted by plasma cells in the spleen and lymph nodes and by cells in the respiratory and gastrointestinal mucosa.

IgE is the only Ig isotype responsible for *atopic (allergic) diseases*. IgE has high affinity for mast cells and basophils. IgE binds these cell surfaces via a specific receptor, termed FcεR. The formation of IgE immune complexes with their specific allergen triggers mast cells to release histamine, serotonin, and other vasoactive amines. IgE is the major component in type I hypersensitivity reactions. While it is not clear why allergens provoke IgE class antibody production in preference to other isotypes, it is likely that the introduction of allergens across mucosal surfaces stimulates Th2-type lymphocytes, which in turn provide help for IgE synthesis. As discussed in the section on cellular immunity, Th2 cells produce interleukin (IL)-4, which drives both IgE and IgG1 production. In experimental systems, the absence of IL-4 decreases the synthesis of IgE and the severity of atopic reactions. As many as 40% of the population have a predilection to produce IgE in response to many environmental antigens.

For more information see Anderson, *IMS: Immunology*. Fence Creek, chapter 9.

3B Antibody-Antigen Interactions

A



B

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J chain	–	–	–	–	+	+	+	–	–
Classical pathway complement activation	++	+	+++	–	+++	–	–	–	–
Opsonization	+++	–	++	+	–	+	+	–	–
Placental transfer	+++	+++	+++	+++	–	–	–	–	–
Binding to macrophages and phagocytes	+	–	+	–	–	–	–	–	+
Sensitization/binding to mast cells and basophils	–	–	–	–	–	–	–	–	+++
Antiviral activity	+	+	+	+	–	+	+	–	–
Bacterial toxin neutralization	++	++	++	++	+	++	++	–	–

All antibody isotypes have a fundamentally similar structure and similar properties for binding antigen (**Part B**). The variable regions within the $F(ab')_2$ region of Ig molecules is aptly named owing to the high amino acid sequence variability at these sites. Three hypervariable regions, called *complementarity determining regions* (CDRs) and named CDR1, CDR2, and CDR3, exist on Ig molecules, with *framework regions* (FRs) located between them. CDRs from both the heavy and the light chain of the Ig molecule confer specificity and affinity to its particular antigen. The collection of multiple combinations of amino acid changes within CDRs on the heavy and light chains is one mechanism by which the immune system generates antibodies of many specificities. In general, the CDRs can be viewed as a mirror image to its specific antigenic determinant—a lock and key–type interaction.

The regions of antigenic proteins that are recognized by antibodies are termed *determinants* or *epitopes*. Epitopes can be based either on the conformational folding of the protein or by short stretches of amino acids called a linear epitope. The ability of short peptides to elicit antibodies is the basis for using synthetic peptides as vaccines against target surface structures of a pathogen.

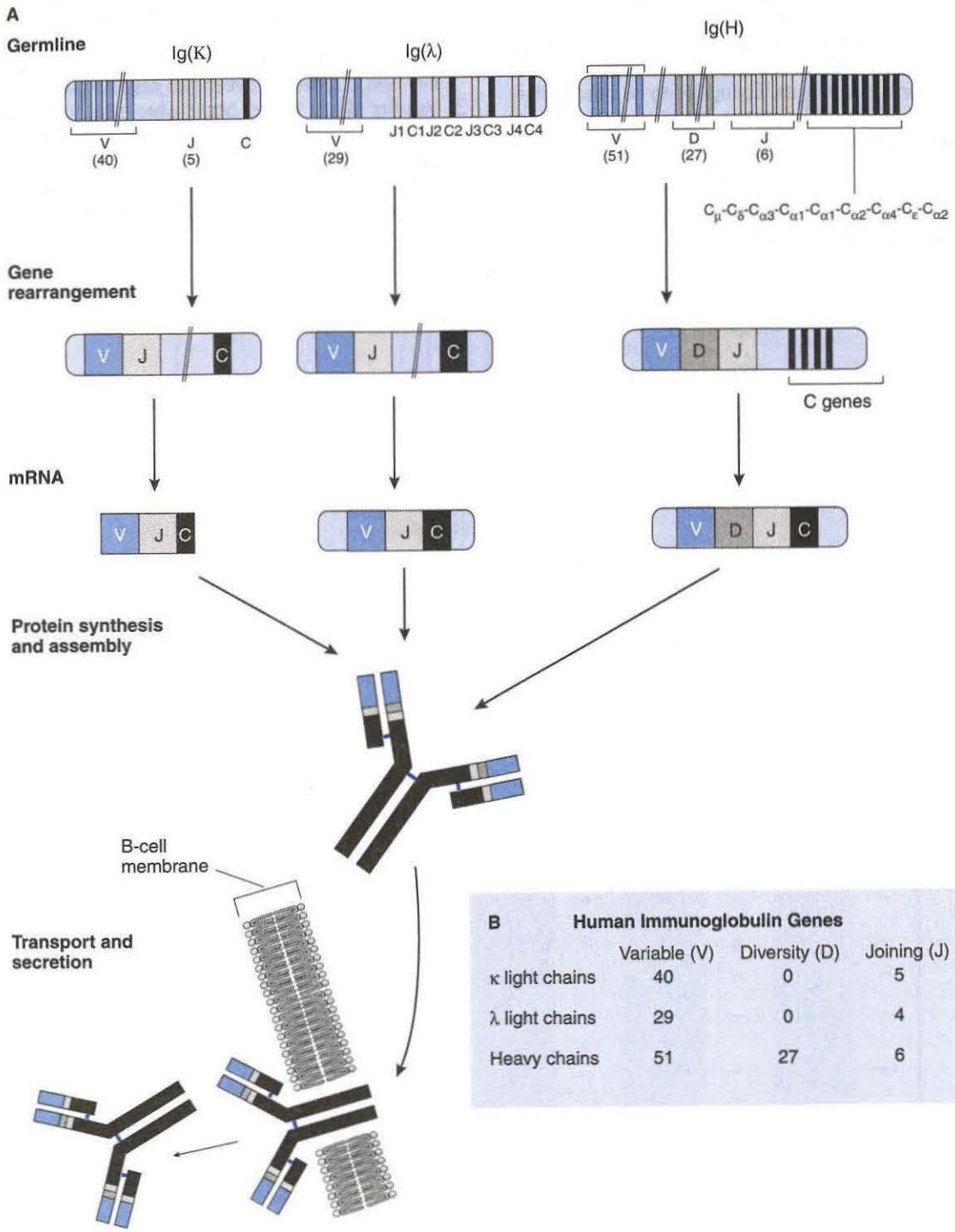
Individuals possess inheritable variants of isotypes, termed *allotypes*, located in the constant regions of heavy and light chains. Over 20 allotype markers exist for IgG heavy chains collectively termed *Gm*. IgA heavy-chain markers are called *Am* while κ light-chain allotypes are termed *Km*.

Several biochemical interactions control the strength of binding of antibody to its antigen. *Idiotype* is the term defining antigen-binding specificity of an individual antibody. Antibodies bind to specific epitopes by 4 principal noncovalent interactions. The combination of all 4 interactions contributes to the overall avidity of an antibody for its antigen:

1. *Electrostatic forces* are interactions generated by the attraction between oppositely charged molecules (positive charges attracted to negative charges).
2. *Hydrogen bonding* occurs when a hydrogen atom with a net positive field is shared between two negatively charged atoms.
3. *Van der Waals forces* occur when sites of electron clouds are attracted between nearby atoms with opposite net polarity.
4. *Hydrophobic interactions* arise when polarized atoms are forced together when surrounded by water molecules. These hydrophobic areas tend to exclude water molecules forcing them together.

For more information see Anderson, *IMS: Immunology*. Fence Creek, chapter 9.

4 The Generation of Immunoglobulin Diversity



B **Human Immunoglobulin Genes**

	Variable (V)	Diversity (D)	Joining (J)
κ light chains	40	0	5
λ light chains	29	0	4
Heavy chains	51	27	6

The success of immune responses depends on the ability of antibodies to form thousands of specificities to account for the vast number of potential antigens encountered in a lifetime. It is estimated that 10^{11} antibody specificities can be generated by the mechanisms that govern the assembly of antibody molecules. While all cells contain germline DNA for antibody genes, only in B lymphocytes are these genes brought together in proximity to allow the transcription of messenger RNA (mRNA) for antibody synthesis.

Both light (L) and heavy (H) chains are assembled from 3 and 4 distinct gene segments, respectively (**Part A**). The light chain is composed of *variable* (V), *joining* (J), and *constant* (C) region gene segments. The heavy chain is composed of V, J, and C gene segments and a *diversity* (D) gene segment. The heavy- and light-chain polypeptides are independently transcribed and assembled inside the B lymphocyte. In the germline, these gene segments are separated by great distances within chromosomes 14 (heavy chain), 22 (λ light chain), and 2 (κ light chain). Somatic rearrangement of DNA brings the V and J region segments of the light chains and the V, D, and J segments of the heavy chain next to one another on all of the 3 chromosomes in the B lymphocyte. As illustrated, a primary mRNA is spliced into a final product that brings all gene segments together as a single contiguous mRNA product (**Part A**). The light-chain polypeptide arises from 2 distinct variable regions of constant genes, κ and λ. In contrast, human heavy-chain constant regions are composed of all the isotypes described earlier, μ (IgM), δ (IgD), the 4 subclasses of γ (IgG_1, IgG_2, IgG_3, IgG_4) the 2 subclasses of α (IgA), and ε (IgE).

Ig diversity arises in part from multiple genes for each V, D, and J segment. The multiple gene segments are located next to one another, with single genes from each segment brought together. For example, 40 separate Vκ region genes exist together with 5 Jκ gene segments. At the heavy chain, 1 of 51 separate V region genes can combine with 1 of 27 different D genes and 1 of 6 J region genes. One quickly realizes how the diversity of antibody specificity can multiply rapidly given the total number of possible gene rearrangements.

Gene rearrangements are strictly controlled by the recombination signal sequence located between all genes. At the junction between gene segments, 7 nucleotides are followed by a spacer sequence of 12 or 23 base pairs, followed by a second group of 9 nucleotides. This *heptamer/nonamer* sequence regulates the precise joining between V, D, and J gene segments on the heavy chain. The gene structure ensures that a V segment will only combine to an appropriate D gene segment and protects against the joining of nonproductive recombination events between multiple V region genes or multiple D region genes, etc.

Mechanisms of Antibody Diversity

Antibodies are able to generate diversity by 4 distinct mechanisms:

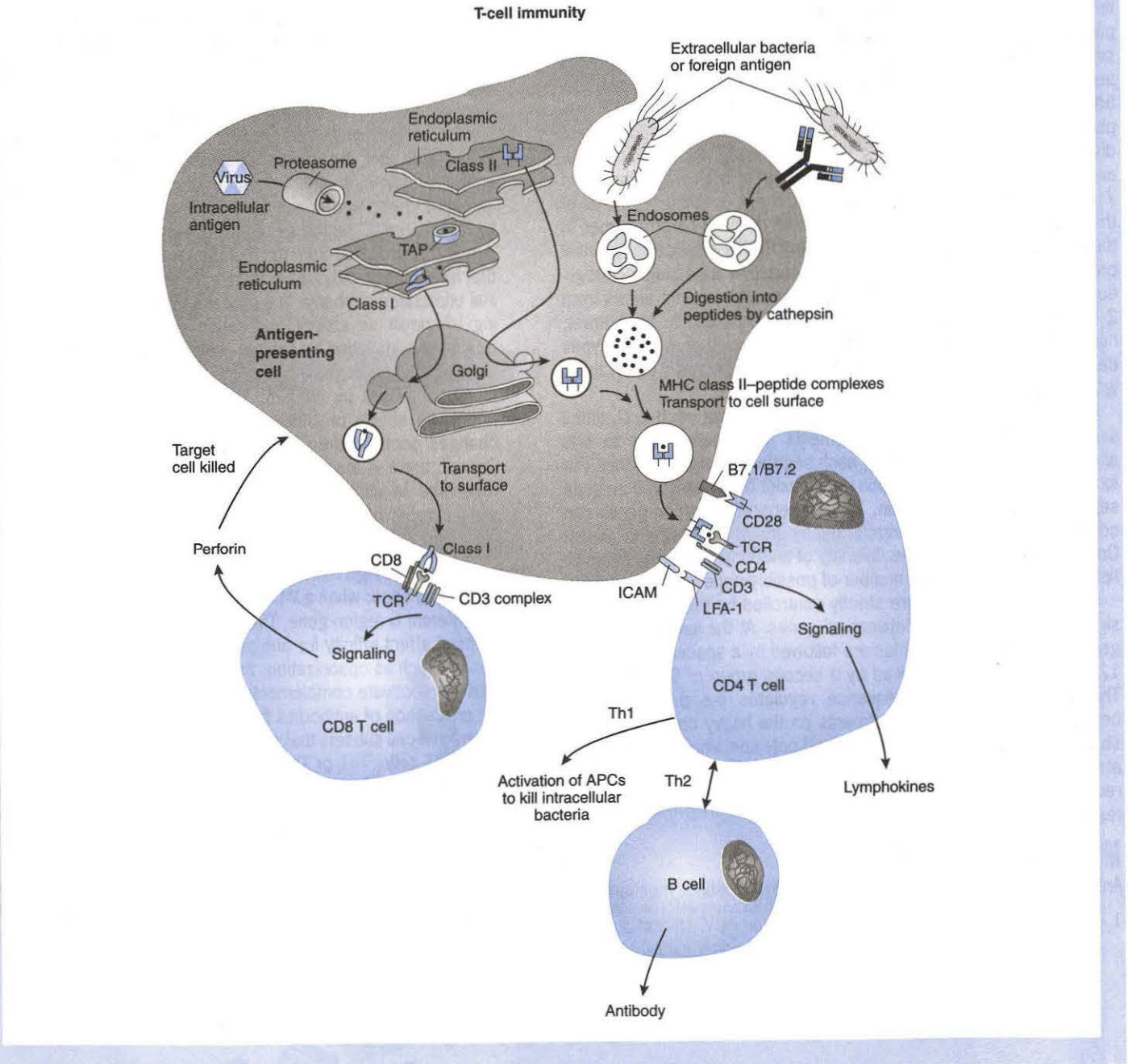
1. As just described, the association of different V, D, and J genes allows for significant variability in the antibodies expressed. For example, 1 of 80 potential variable region genes can combine with any of 5 D region genes or 1 of 6 J region genes for the assembly of the heavy chain.
2. The ability of a single heavy chain to pair with either a κ or λ light chain approximately doubles the number of antibody specificities. However, these interactions may not generate the greatest degree of diversity among these mechanisms, as some species, such as mice, preferentially express κ light chains as opposed to λ light chains.
3. Diversity is generated by differences in individual nucleotide sequences at the joints between the V-D and D-J gene segments. These few nucleotide differences that arise by imprecise recombination processes introduce different amino acid sequences to the translated protein at the junctional regions.
4. Finally, *somatic mutations* arise at selected sites within V region genes that alter the expressed amino acid sequence within the antibody-combining site. Somatic mutation occurs in both heavy and light chains and arises *after* the B-cell receptor is stimulated by its antigen. C region genes do not undergo somatic mutation during this process. Somatic mutation may give rise to antibodies that have significantly higher affinity for antigen than does the original unmutated antibody. The process of enhancing antibody binding is known as *affinity maturation* and occurs over repeated rounds of stimulation by antigen. Affinity maturation enhances the clearance of pathogens, since higher-affinity B cells are most efficiently activated by antigen. The somatic mutations that most affect the affinity of antibody for antigen are when amino acid changes occur in the CDR1 and CDR2 antigen-binding regions. Overall, there is a strong selective pressure to generate a large repertoire of antibodies with high affinity to accommodate the great number of pathogenic antigens exposed to in a lifetime.

Typical antibody responses originate as IgM isotypes followed in time by a switch to IgG isotypes. These changes are known as *isotype switching* and occur when a V-D-J region on the heavy chain combines with a different C region gene. The change in heavy gene usage does not in itself affect affinity for antigen but may enhance other biologic responses such as opsonization, the ability to bind Fc receptors, and the ability to activate complement.

The production of antibodies from plasma cells requires the helper functions of T-cell subsets that are also activated by antigen. It is clear that helper T cells, Th1 or Th2, provide help in the form of cytokines (soluble factors) to B cells in promoting antibody synthesis and secretion. However, some antigens with repeating motifs, such as polysaccharides on bacterial cell walls, can stimulate the production of antibody by B cells without the help of T cells. These antigens are known as thymus-independent (TI) antigens. In contrast, virtually all protein antigens, such as those utilized in vaccines, require T-cell help for antibody production and are known as *thymus-dependent antigens* (TD).

For more information see Anderson, *IMS: Immunology*. Fence Creek, chapter 10.

5 T-lymphocyte Immunity



Stem cells from the bone marrow enter the thymus and undergo several developmental steps, including positive and negative selection, before entering the bloodstream and lymph nodes as mature T lymphocytes. T lymphocytes constitute one important arm of the adaptive immune response, with the other important response being that contributed by B lymphocytes or the humoral immune response. T lymphocytes are divided into 2 major subsets, *cytotoxic T cells (CD8+*, known as *CTLs)* and 2 types of *helper T cells* (both *CD4+* and known as *Th1* and *Th2*).

Cells infected with virus or intracellular bacteria express antigens from these pathogens on their cell surface in the context of major histocompatibility complex (MHC) class I molecules and are directly killed cytotoxic CD8 T cells (**Figure**). T-cell receptors (TCRs) of CD8 T cells bind class I antigen and are stimulated to secrete perforins, which directly lyse the infected cell by introducing pores into the cell membrane in a manner similar to the pores created by the terminal components of the complement cascade.

CD4 T cells recognize antigenic peptide bound to MHC class II mol

ecules (**Figure**). Th1 T cells mediate inflammation and are able to activate macrophages to destroy infectious pathogens. Th1 T cells secrete gamma interferon (IFN-γ), which provides help to B cells that make IgG2. A second class of helper T cells, Th2, secretes interleukin (IL)-4, which activates antigen-specific B cells to produce mainly IgG1 and IgE. Subsets of helper T cells are also defined by the lymphokines they secrete upon activation. Th1 T cells secrete IFN-γ, IL-2, and tumor necrosis factor α (TNF-α). Th2 T cells secrete IL-4, IL-5, IL-6, and IL-10. Please refer to the chapter on lymphokines for specific details of their biologic activities.

Naive T cells exit the thymus into the bloodstream and enter the lymphatic system through high endothelial venules (HEVs). Lymph nodes are the principal site where T cells encounter antigen. T lymphocytes continually circulate through the lymphatics until they encounter the antigen to which they are specific (approximately 1 in 10,000 T cells is specific for a given antigen). Naive T cells that encounter their antigen become activated in a *primary immune response* and initiate effector mechanisms and generate immunologic memory. Long-lived memory T cells are responsible for an accelerated immune response when the same antigen is encountered later. The efficacy of vaccines relies on the generation of memory T cells.

The activation of naive T cells requires two signals from the cell that presents antigen. First, the TCR must bind antigen (*signal 1*) in the context of self MHC class I or II molecules. *Signal 2* is provided by co-stimulatory molecules, B7-1 and B7-2, present on activated antigen-presenting cells (APCs). B7 molecules bind specific ligands on T cells, specifically CD28, which delivers a second signal to activate the T cell (**Figure**). T cells encountering only signal 1 do not become activated but instead become anergic (unresponsive) to the specific antigen. Both CD4 and CD8 T cells require 2 signals for activation. A second receptor for B7 molecules, CTLA4, is responsible for turning off activated T cells. The CTLA4 downregulating molecule prevents T cells from being perpetually activated once antigen is cleared.

Adhesion molecules, such as *LFA-1* on the T cell, bind to *ICAM* molecules on the APCs to enhance cell-to-cell attachment and subsequent activation. T lymphocytes also possess another adhesion molecule, *L-selectin*, that is critical for their homing to specific tissues such as lymph nodes. L-selectin on T cells binds to specific molecules on high endothelial venules or on mucosal endothelium that facilitate their travel into the lymph node.

Activation of Specific T Cells by Antigen

As described previously, secreted antibody or surface Ig of B lymphocytes binds antigens in their native conformation. In contrast, TCRs only bind small peptide antigens that have been degraded inside of APCs and transported to the surface of cells along with MHC class I or class II antigens. T-cell antigenic peptides may originate from intracellular pathogens such as viruses and bacteria that replicate inside of cells. Intracellular pathogens are digested and presented by MHC class I to CD8 T cells. Antigens that originate from extracellular fluids are presented by MHC class II molecules to CD4 T cells. While either self-peptides or foreign peptides may be bound to MHC molecules, T cells have the intelligence to respond only to foreign peptides. T lymphocytes that aberrantly respond to self-peptides may elicit various autoimmune diseases.

Recognition of MHC Class I Peptides by CD8 T Cells

Cells infected with viruses or intracellular bacteria are eliminated by CTLs (CD8 cells). Since virtually all cells can be infected with virus, all nucleated cells also express class I molecules. Nonnucleated red blood cells do not express MHC class I; therefore, intracellular infections within these cells can remain undetectable by CTLs.

MHC class I is composed of 2 polypeptides, α chain and β_2-microglobulin. Viral peptides are generated by protease digestion in an intracellular compartment termed *proteasome*. Viral peptides of 9 to 12 amino acids are transported through pores in the endoplasmic reticulum (ER) membrane composed of TAP-1 and TAP-2 proteins. The peptide forms a stable complex with class I molecules inside the ER lumen, which is then transported to the cell surface.

Recognition of MHC Class II Antigen by CD4 T Cells

The principal role of CD4 T cells is in the activation of B cells and other T cells in the immune system. Only certain cells of the reticuloendothelial system present class II peptide to CD4 T cells. Class II–bearing cells include macrophages, dendritic cells, specialized APCs in lymph nodes, and B cells. Other nucleated cells express little or no class II. Peptides that become associated with class II originate in acidic endosome vesicles inside the cell. Antigen may be engulfed from the extracellular fluids and be retained inside acidified endosomes for degradation into peptides. Alternatively, some intracellular bacteria or parasites may replicate inside these acidic vesicles and become a source of peptides for MHC class II. B lymphocytes internalize extracellular antigens or pathogens by binding to their surface Ig. In either case, the vesicle experiences a decrease in pH, which activates proteases, including *cathepsins B, D,* and *L*. These proteases digest antigens within the vesicle into 12 to 20 amino acid peptides for assembly with class II.

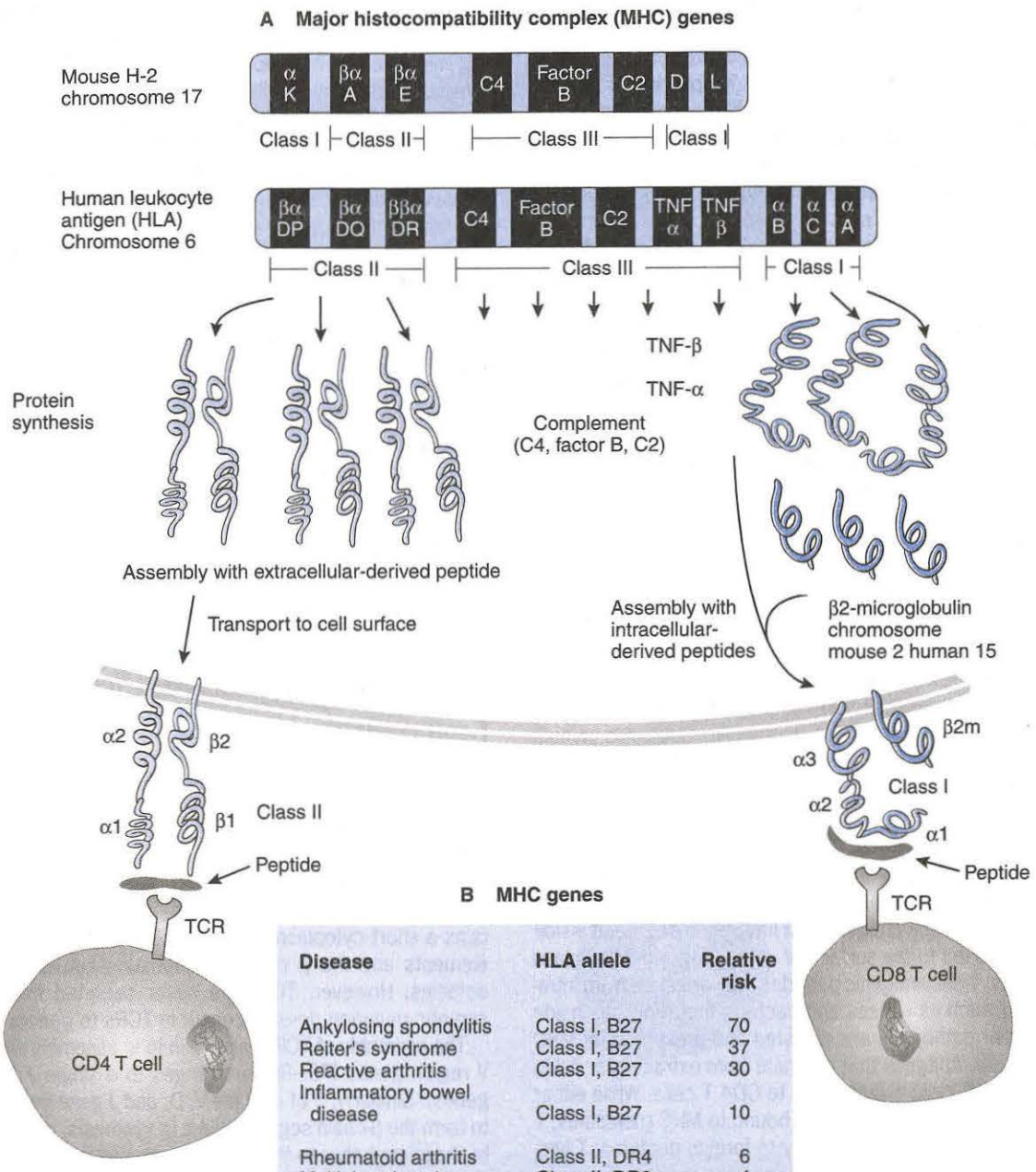
The class II molecule is composed of 2 noncovalently associated polypeptide chains. Overall, the structure of class I and class II molecules is quite similar in that the peptide presented is within a groove created by the 2 polypeptide chains. Binding to either type of MHC molecule occurs by only a few of the total amino acids of the polypeptide. Three to four anchor residues control binding to the MHC molecule by noncovalent interactions, including hydrophobic interactions and charge interactions. Remember that it is important for the MHC molecules to present thousands of different peptide motifs. Likewise, only a few of the amino acid residues extend above the peptide binding groove and mediate binding by the TCR.

T-cell Receptor

The TCR encounters the same requirements for generating many specificities as do the B-cell receptor and antibody molecule. The T cell will encounter virtually limitless numbers of antigens in its lifetime. The TCR closely resembles the $F(ab')_2$ fragment of Ig anchored to a T-cell membrane. Like Ig, the TCR is composed of 2 noncovalently associated chains, α and β. Each chain spans the membrane and contains a short cytoplasmic domain. The α chain contains V- and J-like elements and the β chain has domains consisting of V, D, and J domains. However, TCRs are never secreted from the T cell and somatic mutation *does not* occur in TCRs to generate diversity.

The assembly of TCR is also akin to Ig assembly where 1 of multiple V region genes (70–80) rearranges to a single J gene (>60 known genes). Similarly, 1 of multiple V, D, and J gene segments rearranges to form the β-chain segment. Like Ig synthesis, nucleotides are added to the joints between the V and J gene segments, adding to diversity. Finally, signaling through the TCR is accomplished through accessory molecules, called the *CD3* complex. As the TCR engages antigen-MHC complexes, the CD4 and CD8 molecules enhance this association. CD4 and CD8 bind to sites on MHC class II and class I, respectively, and serve to enhance T-cell activation. The presence of CD4 or CD8 reduces the amount of antigen required to activate the T cell by nearly 100-fold.

6 Major Histocompatibility Gene Complex (MHC)



B MHC genes

Disease	HLA allele	Relative risk
Ankylosing spondylitis	Class I, B27	70
Reiter's syndrome	Class I, B27	37
Reactive arthritis	Class I, B27	30
Inflammatory bowel disease	Class I, B27	10
Rheumatoid arthritis	Class II, DR4	6
Multiple sclerosis	Class II, DR3	4
Systemic lupus erythematosus (SLE)	Class II, DR2	3
Myasthenia gravis	Class II, DR3	3
Hashimoto's thyroiditis	Class II, DR5	3
Type I diabetes (IDDM)	Class II, DR3/DR4 (heterozygote)	5

Major histocompatibility complex (MHC) proteins are antigens originally defined in studies of tissue transplantation. It was observed that some tissues could be engrafted or rejected between strains of mice based on surface proteins later to be defined as MHC. MHC antigens are the major cause of immune-mediated rejection responses in tissue transplantation (for details, see chapter on transplantation). In humans, the MHC proteins are termed *human leukocyte antigens* (HLA), as they are found in high concentrations on white blood cells as well as other nucleated cells in the body. The principal HLA antigens are termed *class I, class II,* and *class III*. The genes for HLA antigens are found on the short arm of chromosome 6 in humans. The MHC antigens in mice (termed *H-2*) are found on chromosome 17. One component of the class I molecule, β_2-microglobulin, is found on human chromosome 15 and on mouse chromosome 2.

The principal function of MHC molecules is to present peptide fragments from foreign antigen for recognition by and activation of appropriate T-cell subsets in the adaptive immune response. The end product of this MHC peptide interaction is in initiating a full immune response of B and T lymphocytes, the release of cytokines, and the recruitment and activation of other phagocytic cells such as macrophages. The ability to clear and destroy pathogens is based on the ability of MHC molecules to bind and present peptides of the pathogen. The properties of MHC proteins allow them to bind a wide range of different peptides, making it difficult for pathogens to escape immune responses. MHC proteins are defined as *polymorphic*; many alleles of each gene are present in the genome, allowing a greater variety of peptide specificities to be bound and presented. The combination of MHC genes found within an individual chromosome is termed MHC *haplotype*. Finally, individuals are often *heterozygous* for individual loci, meaning that each chromosome encodes a different MHC specificity, which are presented on the surface of the cell. The translation and presence of both genes at a loci is termed *co-dominant expression*.

Three distinct class I genes exist in humans: HLA-A, -B, and -C. Again, proteins at each locus are capable of binding distinct types of peptides for presentation. In humans, 41 different alleles have been identified at the A locus. For the B and C locus, 71 and 27 different alleles have been defined, respectively. Class I proteins are present on all nucleated human cells and on platelets (red blood cells do not have MHC antigens). Class I antigens are composed of 2 chains, one 44-kd α chain with 3 individual domains (α1, α2, α3) and a second chain composed of *β_2-microglobulin*. The 2 chains are noncovalently assembled inside the cell and transported to the surface with peptide for presentation to CD8 T cells. The CD8 T-cell receptor only recognizes foreign peptide in the context of class I MHC proteins. Class I peptides are processed and presented from intracellular proteins or pathogens such as intracellular virus.

Class II antigens are present primarily on reticuloendothelial cells (cells only of the immune system) but not on other nucleated cells in the body. Class II is found on B and T lymphocytes, macrophages, and monocytes. Three types of class II genes also exist in humans: HLA-DR, HLA-DQ, and HLA-DP. All of them bind peptide for presentation to CD4 T cells. As for class I antigens, many different class II DP, DQ, and DR alleles have been identified in humans (**Part A**). An individual may possess 2 different proteins for each locus on the cell surface, one from each chromosome (co-dominance). Class II antigens are composed of 2 polypeptide chains, α and β, each with 2 domains (α1, α2 and β1, β2, respectively). The 2 chains are assembled with a class II peptide fragment and transported to the cell surface for presentation to CD4 T-cell receptors. Normally, antigen-presenting cells (dendritic cells, macrophages, and B lymphocytes) present class II peptide complexes to T cells. CD4 T cells activated in this manner are stimulated to secrete cytokines and to provide help for antibody synthesis by β lymphocytes (see chapter on cytokines). Class II antigenic peptides are processed and presented from extracellular proteins or pathogens that are phagocytized into antigen-presenting cells.

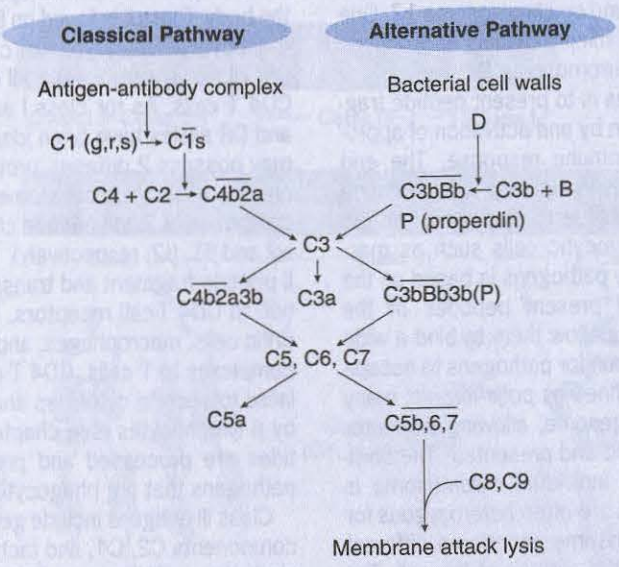
Class III antigens include genes that transcribe complement protein components C2, C4, and factor B. The genes encoding tumor necrosis factor α (TNF-α) and TNF-β are also found within this site. These proteins are not formally involved with histocompatibility (transplantation rejection) but have historically been called MHC class III antigens.

The presence of individual MHC alleles can often predict susceptibility to certain diseases, as shown in **Part B**. For example, the class I allele B27 is found in high frequency in patients with ankylosing spondylitis. Please refer to chapter on autoimmunity for details.

For more information see Anderson, *IMS: Immunology*. Fence Creek, chapter 8.

7 The Complement System

A



The complement cascade

B

Complement Component	Biologic Activity
C3a, C4a, C5a	**Anaphylotoxins** that mediate release of histamine, serotonin,and vasoactive compounds from mast cells and basophils. Inflammation, smooth muscle contraction, and vascular permeability are increased.
C3b, iC3b, C4b	**Cause opsonization and adherence** by linking immune complexes to phagocytic cells (macrophages and neutrophils) via membrane C' receptors. Erythrocytes bearing C' receptors enhance clearance of immune complexes through the liver and spleen.
C5a	**Causes chemotaxis**; attracts macrophages and neutrophils to the site of C' activation, thereby enhancing phagocytosis.
C8, C9	**Cause membrane disruption**, resulting in the osmotic lysis of cells.
Ba	**Causes chemotaxis** for neutrophils.

Serum complement was originally identified as a heat-sensitive component of serum that enhanced both innate and adaptive immune responses. These functions include an influence on inflammation reactions as well as the opsonization and eventual killing of bacteria by antibody together with end-stage complement components. Moreover, certain cells of the immune system bear complement receptors, allowing them to engulf and kill complement-bound bacteria. The complement system represents a collection of over 15 serum proteins, only one of which actually binds antibody molecules. Thereafter, a cascade of subsequent protein interactions leads to destruction of the infected cell or pathogen. Complement proteins, designated as "C," are defined as MHC class III proteins whose genes are found on chromosome 6 in the human. The liver is a major source of serum complement proteins.

Two principal pathways exist for the biologic activation of complement: the *classical pathway* and the *alternative pathway* (**Part A**). The classical pathway is activated by the binding of antibody to antigen such as in immune complexes or in the binding of antibody to tumor cells or virally infected cells.

Classical Complement Pathway

The formation of antigen-antibody complexes initiates the classical pathway and is therefore an important feature of the adaptive immune response. Complement is activated by IgM and all isotypes of IgG with the exception of IgG4. IgA, IgE, and IgD do not activate complement. The order of complement protein activation is *1, 4, 2, 3, 5, 6, 7, 8, 9*. C1 is a trimolecular complex of three proteins, C1q, C1r, and C1s, and is the first activated protein to bind to the Fc region of IgG or IgM (in particular, the CH2 domain of IgG or the CH3 domain of IgM). C1q can bind to a single pentameric IgM-antigen complex but requires 2 or more IgG antibody-antigen complexes for binding, owing to the monomeric structure of IgG. The pentameric structure of IgM accounts for its enhanced ability to initiate complement activation. C1q bound to the Ig molecule initiates activation of the C1r proenzyme and subsequent activation of C1s. Activated C1s is able to cleave 4 molecules of C4 into C4a and C4b in a first amplification step of the complement cascade. C4 is an anaphylatoxin causing the release of vasoactive amines from mast cells. The next complement protein, C2, is cleaved into C2b and complexes with C4b. C2a is released from the complex. $\overline{C4b2b}$, a serine protease termed C3 convertase, cleaves C3 into C3a and C3b. C3a is yet another anaphylatoxin while C3b has several biologic functions and is therefore a key complement protein in the pathway. C3b is a critical component in forming $\overline{C4b2b3b}$, a complex that acts on C5. Additionally, C3b is one of the initiating components in the alternative C′ pathway (see below).

C5 is the step at which the classical and alternative pathways converge. C5 convertase ($\overline{C4b2b3b}$ or $\overline{C3bBb}$ from the alternative pathway) cleaves C5 into C5a and C5b. C5a, the third anaphylatoxin in the path, serves to increase vascular permeability while C5b binds C6. Thereafter, C7 binding forms a stable complex (C5b67). C5b67 attaches to the target cell, although no cell damage or lysis occurs until attachment by C8 and C9. C8 and C9 are the terminal complement components forming the membrane attack complex (MAC). While C5b678 complexes can form small pores in the cell membrane, the polymerization of C9 punches large channels in the lipid bilayer membrane, causing the osmotic lysis of the cell. The C9 terminal component requires the presence of divalent cations, such as Mg^{2+}, for binding to the complex. Cells nearby are protected from inadvertent lysis since the MAC is extremely labile. The MAC resembles the structure and function of perforin, a molecule that also lyses cells and is synthesized by cytotoxic T cells.

The Alternative Pathway

The alternative pathway is important in the *innate* defense of microorganisms. The pathway is triggered either by bacterial membrane components, lipopolysaccharides (LPS) and teichoic acids; by yeast cell walls; or by some parasites. The alternative pathway is initiated by the C3 component and does not require C1, C4, or C2.

As a first step in this pathway, C3b is bound by serum factor B. This complex allows factor D, a serine protease, to cleave factor B into Ba and Bb, now termed $\overline{C3bBb}$. The Ba peptide is released and attracts neutrophils to the site (a chemoattractant). It is important to remember that downregulatory proteins H and I can bind C3b and inhibit subsequent activation events. This mechanism prevents unrestricted or uncontrolled activation of this pathway. Conversely, the pathway can be amplified, when necessary, by the binding of properdin to $\overline{C3bBb}$. This stable complex cleaves additional C3 to C3b. $\overline{C3bBb}$ is a C5 convertase, acting to cleave C5 to C5a and C5b. The remaining steps are identical to those of the classical pathway.

Regulation and Amplification of the Complement Pathways

Innate immunity must act quickly in the defense against microorganisms while the adaptive response evolves to develop specific antibodies or cellular immunity. For this reason, key steps in the complement pathway can be amplified quickly. For example, C1s, a serine protease, cleaves many C4 molecules. At the next step, a single membrane-bound C4b molecule cleaves several C2 proteins. A major amplification step occurs when the $\overline{C4b2b}$ complex acts on thousands of C3 molecules. Likewise, in the alternative pathway, properdin-$\overline{C3bBb}$ cleaves thousands of C3 proteins. As indicated earlier, membrane-bound C3b interacting with factors B and D forms a positive feedback loop in clearing additional C3 molecules. These amplification circuits expedite the attack on microorganisms, before they overcome the host.

Biologic Roles of Complement Components

In addition to the direct lytic functions of the terminal complement components, C8 and C9, other complements function to promote the clearance of pathogens (**Part B**). These functions are broken down into 3 central roles:

1. **Anaphylatoxin.** C3a, C4a, and C5a are molecules that degranulate mast cells, causing the release of histamine and other vasoactive amines. The increase in vascular permeability allows a greater influx of cells to the site of infection. Mast cells and basophils bear surface receptors for C3a and C5a, two anaphylatoxins in the complement cascade. Receptor binding by either C3a or C5a causes the release of inflammatory vasoactive amines (histamine). C3a, C4a, and C5a increase vascular permeability, causing inflammation and smooth muscle contraction.
2. **Opsonization.** Opsonization is mediated by receptors for complement products on macrophages and neutrophils that enhance phagocytosis. Pathogens bound by C′ are readily engulfed by cells bearing complement receptors. C3b binds directly to the surface of pathogens and may then bind to complement receptor 1 (CR1) found on macrophages, monocytes, peripheral mononuclear cells, and B cells. Three other receptors, CR2, CR3, and CR4, bind inactivated C3b (termed iC3b), which remains bound to the surface of pathogens.
3. **Chemotaxis.** Phagocytic cells are attracted to the sites of inflammation to aid in the clearance of microorganisms. C5a is the major chemoattractant drawing macrophages and neutrophils to the site of complement activation. In addition, C5a serves to activate and enhance the adherence of neutrophils to vascular endothelium.

For more information see Anderson, *IMS: Immunology*. Fence Creek, chapter 14.

8 Cytokines and Chemokines

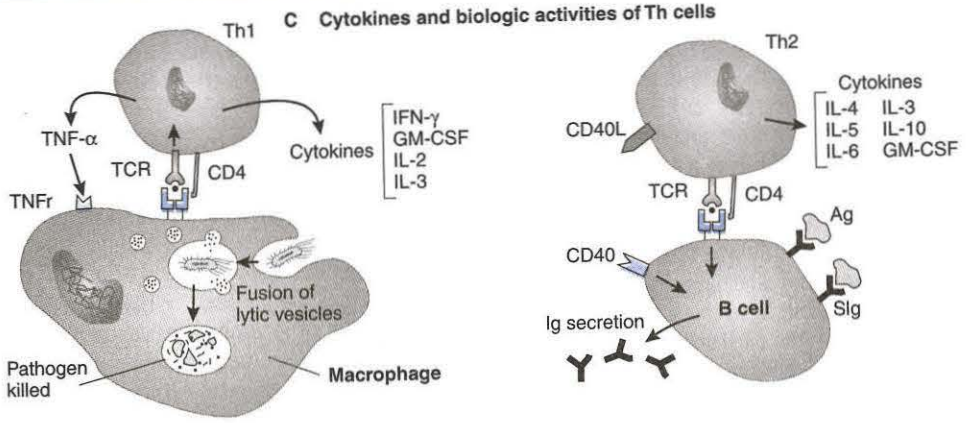
A Table I

Cytokine	Source	Biologic Activity
IL-1	Macrophages, epithelial cells	Macrophage and T-cell activation, induces fever
IL-2	Th1 cells	T-cell growth/activation
IL-3	Th1, Th2, cytotoxic T-cells, thymic epithelial cells	Hematopoiesis
IL-4	Th2 cells	B-cell activation, IgG1, IgE synthesis
IL-5	Th2cells	Eosinophil proliferation
IL-6	Th2 cells, macrophages	T-cell, B-cell growth and differentiation
IL-7	Bone marrow	pre-T-cell and pre-B-cell growth
IL-9	T cells	Mast cell growth
IL-10	Th2 cells, macrophages	Suppresses macrophage function
IL-11	Fibroblasts	Helps IL-3, IL-4 in hematopoiesis, increases MHC class II
IL-12	B cells, macrophages	Enhances Th1 differentiation, activates NK cells
IL-15	T cells	Epithelial cell growth
GM-CSF	Th1 cells, fibroblasts	Neutrophil stimulation and growth
IFN-α	Leukocytes	Antiviral, enhances MHC class I expression
IFN-γ	T cell, NK cells	Macrophage activation, enhances MHC protein expression
TNF-α (cachectin)	Macrophages, NK cells	Inflammation, endothelial cell activation
TNF-β (lymphotoxin, LT)	T cells, B cells	Targets cell killing
TGF-β	Monocytes, T cells, chondrocytes	Antiinflammatory

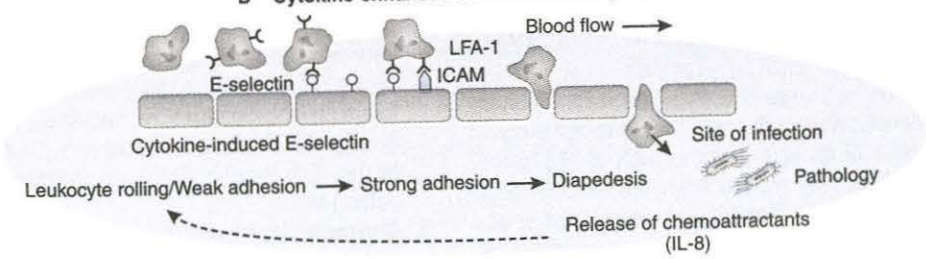
B Table II

Chemokine	Source	Biologic Activity
IL-8	Macrophages, fibroblasts, keratinocytes	Chemotactic for T cells and neutrophils
MCP-1	Macrophages, fibroblasts, keratinocytes	Monocyte chemotaxis and activation
MIP-1α	Macrophages	Attracts eosinophils, T cells, and monocytes
MIP-1β	Monocytes, T cells, B cells	Attracts T cells, monocytes
RANTES	T cells, platelets	Attracts monocytes, T cells, and eosinophils

C Cytokines and biologic activities of Th cells



D Cytokine-enhanced cell adhesion/diapedesis



Cytokines and chemokines are soluble proteins secreted by a cell that directly changes the biologic properties of cells like itself or other cell types. Cytokines (**Part A**) may be made by lymphoid cells or some cells outside the lymphoid system such as fibroblasts and epithelial cells. In general, *cytokine* denotes the collection of bioactive proteins made by all of the cell types. Macromolecules secreted by lymphoid cells are termed *lymphokines*, with those produced by T lymphocytes denoted numerically as *interleukins* (ILs). Cytokines either exhibit biologic activity within a local cellular milieu or sometimes have systemic biologic effects far from their source of synthesis. These properties are based in part on the kinetics of receptor expression on various cells. The expression of cytokine receptors on specific target cell types further focuses the biologic effects of these proteins. Chemokines (**Part B**), which recruit cells to sites of inflammation (chemoattractants), tend to have systemic rather than local biologic effects. Hematopoietic cytokines such as IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) also act systemically.

Many of the cytokines have multiple or overlapping biologic functions to the cells targeted for activity. For example, both IL-2 and IL-4 enhance growth and survival of T lymphocytes. Cell populations can be defined by the sets of cytokines produced. CD8 cytotoxic T cells (CTLs) secrete interferon (IFN)-γ, which has direct antiviral properties. In contrast, IFN-γ secreted by Th1 cells promotes IgG2 and IgG3 synthesis by B lymphocytes. A number of the cytokines attract phagocytes and enhance vascular permeability, properties that are also shared by activated complement components, C3a and C5a. These interacting functions create a synergy between bioreactive macromolecules at the site of infection.

The *innate immune response* relies on the biologic activity of cytokines for the recruitment of phagocytic cells to the site of infection. Macrophages that become activated by pathogens release IL-1, IL-6, IL-8, and IL-12 as well as tumor necrosis factor (TNF)-α, all of which enhance inflammatory responses by the attraction or activation of lymphoid cells. Pain, redness, heat, and swelling are signs of active infection and are responses amplified by cytokines. IL-1 activates lymphocytes and endothelial cells in the vessels, allowing a greater influx of other macrophages and T lymphocytes. A by-product of IL-1 and IL-6 release is fever.

TNF-α has important local and systemic effects. Locally, TNF-α increases vascular permeability and the influx of plasma fluids (i.e., swelling), phagocytic cells, and Ig, which carries the pathogen to draining lymph nodes for presentation to B and T cells. Local blood clotting is enhanced, which impedes the spread of infection to the bloodstream. If the pathogen invades the bloodstream, termed *sepsis*, systemic TNF-α can be lethal. TNF-α–mediated systemic vasodilation leads to *disseminated intravascular coagulation* (DIC) and massive loss of plasma fluids into the tissue spaces. Septic shock causes the failure of major organs including the kidney, lungs, and liver.

IL-8 and TNF-α enhance the expression of vascular adhesion molecules, E-selectin, P-selectin, and ICAM. Selectins on the surfaces of activated endothelium bind to the receptors of circulating macrophages and neutrophils (sialyl Lewis^x) and direct their emigration through the vessels into the tissue spaces. Cells are reversibly bound by selectin and continue rolling along the vasculature until stronger binding interactions take place between the integrins, LFA-1 and ICAM. Integrins are a family of cell surface proteins that mediate adhesion and homing of cells to tissues. As affinity of integrin interaction increases, phagocytic cells stop rolling along the vessels and migrate across the epithelial spaces and to the site of infection, called *diapedesis* (**Part C**).

The success of adaptive immune responses also depends on the role of cytokines, most apparent by the ILs synthesized by helper T-cell subsets. Th1 and Th2 helper T cells (**Part D**) drive the production of specific antibody isotypes, provide growth and activation molecules for other T cells and macrophages, and promote the synthesis of MHC molecules that present foreign antigen. Th1 cells produce IFN-γ, GM-CSF, and TNF-α. These cytokines promote inflammation, as described, in addition to activating macrophages to kill pathogens they have engulfed or that have infected them. GM-CSF has the systemic effect of stimulating myelopoiesis in the bone marrow.

Th2 cells primarily secrete cytokines that enhance the adaptive immune response of B lymphocytes through antibody production. IL-4, IL-6, and the Th2 cell surface protein CD40L promote B-cell activation and the production of IgG1 and IgE. IL-4 also stimulates the synthesis of MHC class II molecules for the presentation of antigen by antigen-presenting cells.

It is clear that the innate and adaptive immune response to a pathogen is a highly orchestrated interaction of cytokines, which have effects on vascular tissues and on the expression of synergistic cell surface molecules (i.e., MHC and adhesion molecules), and stimulate the growth and attraction of immune cells.

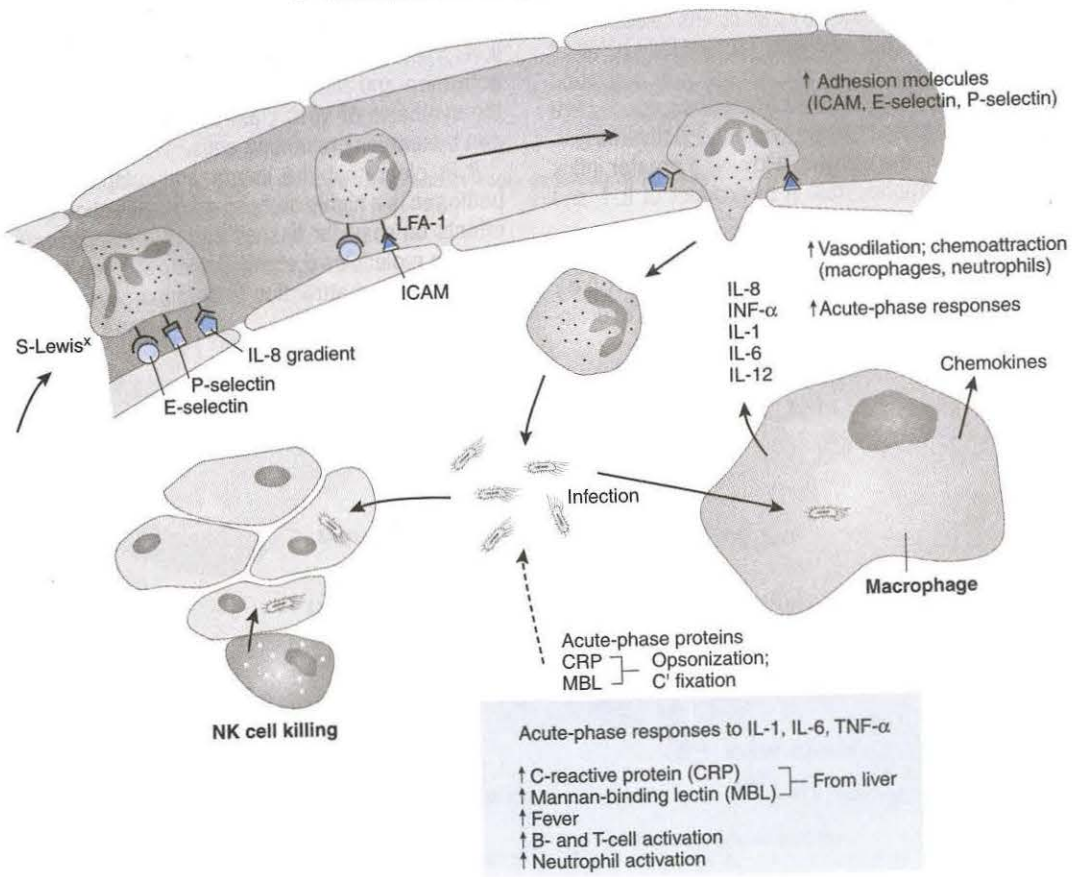
For more information see Anderson, *IMS: Immunology*. Fence Creek, chapter 6.

9A Innate Immune Responses to Infection

A Table I

Compound	Activities	Local and Systemic Effects
TNF-α	↑ Vasodilation; ↑ Epithelial adhesion molecules (E-selectin, P-selectin, ICAM-1)	↑ Influx of fluids, Ig, C' proteins; binding and extravasation of phagocytes; induces acute-phase responses
IL-1	↑ B- and T-cell activation; ↑ IL-6 production	Fever; tissue destruction
IL-6	B- and T-cell growth, activation	Induces acute-phase responses; fever
IL-8	↑ Adhesion; granulocyte activation	Leukocyte chemotaxis
C-reactive protein (CRP)	Binds bacterial and fungal surfaces	Opsonization and C' activation
Mannan-binding lectin (MBL)	Binds bacterial surface mannose	Opsonization and C' activation
Ifn-α/Ifn-β	↑ MHC class I; activates NK cells	Antiviral

B Innate Immune Responses to Infection



The immune system evolved to protect the host from infectious agents. In this role, immunity takes two forms, the first being controlled by so-called *innate* or *nonadaptive* immune responses, followed by a second phase termed *adaptive* immunity. The role of the innate immune response is largely to control or prevent the initial spread of pathogens while the adaptive immune response develops. Phagocytic cells are the first line of defense in the immune system. In addition, cytokines that are released by phagocytes attract other cells to the site of infection. Cytokines released in this response include IL-1, TNF-α, IL-6, IL-8, and IL-12 as well as a host of chemokines and acute-phase proteins. **Part A** lists biologic compounds involved in the innate immune response, and their activities and effects.

Inflammation is an important immediate component of the innate immune response. Other inflammatory compounds released by phagocytes early in infection include *nitric oxide*, *oxygen radicals*, *prostaglandins*, *leukotrienes* (*LTB_4*), and *platelet-activating factor* (*PAF*). The activation of complement proteins C3a and C5a by the alternative pathway also contributes to inflammation. C5a can directly activate and degranulate mast cells, causing the release of histamine and LTB_4, resulting in vasodilation. All of these mediators contribute to the characteristic early signs of inflammation due to infection, including swelling, redness, heat, and pain.

Shortly following these immediate inflammatory responses, the next effect of these soluble cytokines is to enhance the expression of adhesion molecules, *E-selectin*, *P-selectin*, and *ICAM-1*, on endothelial cells near the site of infection (**Part B**). These adhesion molecules allow circulating leukocytes to adhere and extravasate through the vessel walls into the tissue. The first adhesion molecule, called P-selectin, is stimulated immediately onto the surface of endothelial cells after exposure to LTB_4, C5a, or histamines. P-selectin is maintained inside of endothelial cells in granules called Weibel-Palade bodies. Thereafter, a second important adhesion molecule, E-selectin, is stimulated by lipopolysaccharide (LPS) or TNF-α. Circulating monocytes and neutrophils bind to P- and E-selectins, rolling across the vessel surface and causing more tightly adhesive interactions to occur. The strongest interactions occur when LFA-1 on the leukocyte interacts with ICAM-1 expressed on the endothelial cell after exposure to TNF-α. The final step of this interaction allows the leukocyte to cross the endothelial barrier through junctions between cells that have been created by histamines and other vasoactive agents.

Tumor Necrosis Factor (TNF)-α in Innate Immunity

TNF-α is an important mediator of innate immune responses in infection. TNF-α has the ability to initiate the blood-clotting cascade in small vessels, thereby preventing the spread of bacteria into the bloodstream and to other parts of the body. This mechanism helps contain the pathogen at its initial site of infection. However, should the infection become blood borne (sepsis), the systemic release of TNF-α from macrophages at other sites (liver and spleen) can cause systemic vasodilation and the loss of plasma fluids, leading to septic shock and possibly death. Disseminated intravascular coagulation initiated by systemic TNF-α causes the failure of several important organs such as the kidneys, heart, lungs, and liver.

Chemokines

Chemokines are small proteins synthesized by several cell types including fibroblasts, smooth muscle cells, endothelial cells, and phagocytes. The main function of chemokines is to attract phagocytic cells, primarily monocytes and neutrophils, to the initial site of infection. Chemokine synthesis can be initiated in many ways including tissue trauma, bacterial products, and viral infections. Some chemokines including *IL-8* not only attract neutrophils, but also activate them for the immediate attack of pathogens. This function is performed by the activation of toxic oxygen radicals and nitric oxide inside of phagocytes.

IL-8 and MCAF also recruit cells to a specific site by concentration gradients that increase toward the site of infection. This allows the adhesion interactions of leukocytes on endothelial cells to migrate in the direction of the infection. IL-8 and MCAF bind to endothelial cell surfaces, acting as a road map for the leukocytes to follow. After extravasation of the leukocytes through the endothelium, they then follow the concentration of chemokines to the specific site of infection.

Innate Mechanisms That Clear the Pathogen

Neutrophils are important early phagocytic cells in the clearance of pathogens. Although neutrophils survive only several hours after leaving the bone marrow, they quickly migrate to the site of infection where their phagocytic properties begin clearing bacteria. Neutrophils are capable of engulfing either free bacteria or opsonized (antibody or complement-coated) bacteria. Some bacterial cell wall compounds, including LPS, can be bound by neutrophil surface components, thereby facilitating uptake into the cell. Finally, neutrophils also can actively engulf complement-coated bacteria by virtue of C3b receptors on its cell surface. However, polysaccharide-encapsulated bacteria are resistant to direct phagocytosis and usually require opsonization by antibody for phagocytic clearance.

Neutrophils produce a variety of compounds that are bacteriostatic or that kill the internalized pathogen. These include toxic oxygen radicals, nitric oxide, phospholipases, proteases, and compounds able to kill bacteria, fungi, and some enveloped viruses. The important role of neutrophils in innate defense is underscored by the presence of recurrent bacterial and fungal infections in patients who have neutrophil deficiencies (see chapter on immune deficiency syndromes). Finally, the pus that forms at the site of infections is largely composed of neutrophils that have engulfed bacteria and died. These cells are replaced by new incoming neutrophils and other phagocytic cells such as macrophages to further control the infection.

For more information see Anderson, *IMS: Immunology*. Fence Creek, chapter 2.

9B Innate Immune Responses to Infection

A Table I

Compound	Activities	Local and Systemic Effects
TNF-α	↑Vasodilation; ↑Epithelial adhesion molecules (E-selectin, P-selectin, ICAM-1)	↑ Influx of fluids, Ig, C' proteins; binding and extravasation of phagocytes; induces acute-phase responses
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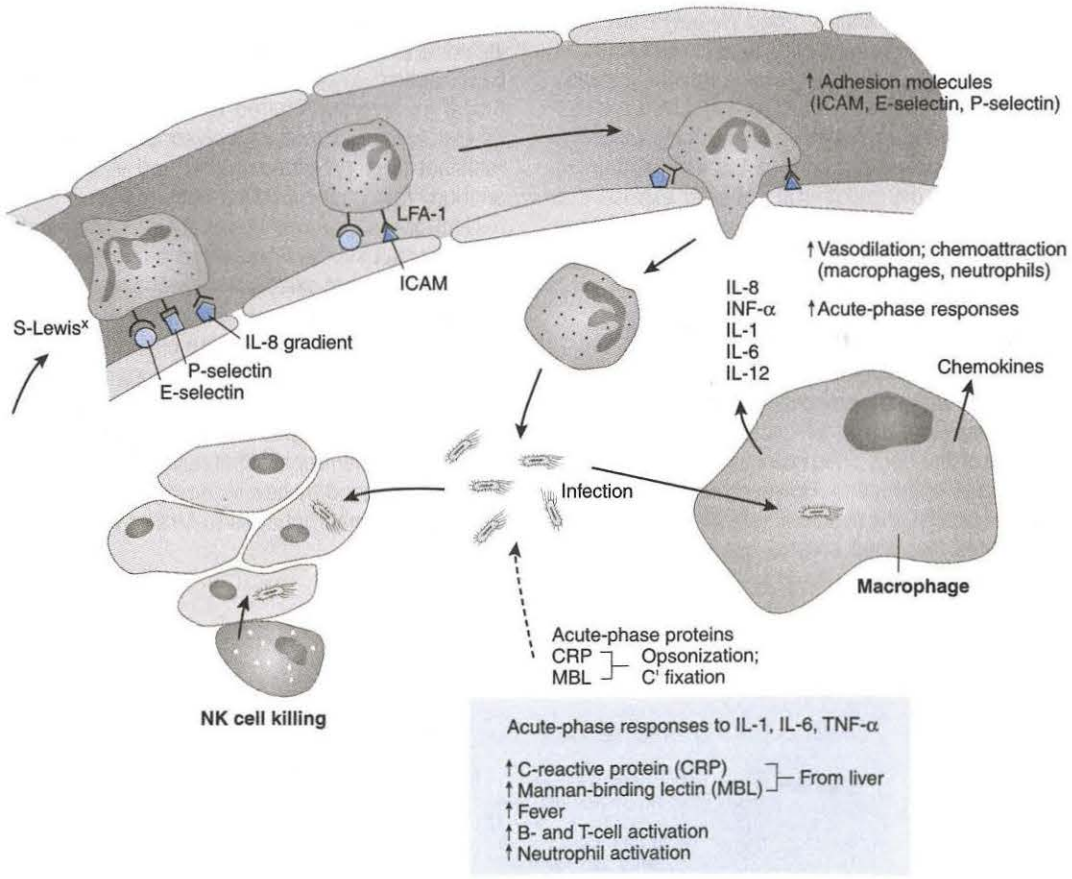
B Innate Immune Responses to Infection



Acute-Phase Responses

Fever is a well-described indication of ongoing infection and is considered protective because many pathogens do not grow well at high body temperatures. The increase in body temperature can be caused by a number of factors including TNF-α, IL-6, and IL-1. Whereas bacterial products themselves raise body temperature, cytokines produced by infection are termed *endogenous pyrogens* for their ability to induce fever. Cytokines also induce the production of acute-phase compounds in the liver. Two important acute-phase proteins produced in the liver are called *C-reactive protein (CRP)* and *mannan-binding lectin (MBL)*. CRP has important antibacterial properties, owing to its ability to directly bind to bacterial and fungal cell wall LPS. CRP also activates the conventional complement pathway to further aid in clearance of the pathogen. In a similar manner, MBL binds to mannose residues on the surfaces of many bacteria. MBL can act as an opsonin aiding in phagocytosis by monocytes. These acute-phase proteins are important in innate immunity because of their ability to bind a great variety of bacterial pathogens.

Interferon in Innate Immunity

Soluble cytokines are also critical in early responses to infectious agents. In particular, the interferon group of cytokines have important antiviral properties. Interferons were first identified by their ability to interfere with viral growth and replication in culture. There are 3 types of interferons: IFN-α, IFN-β, and IFN-γ. IFN-α and IFN-β are synthesized and secreted by many cell types such as fibroblasts and epithelial cells, while IFN-γ is a product of activated T cells. Viral infection stimulates the production of IFN-α and IFN-β, which bind to specific cell-surface receptors, inducing them to make several intracellular macromolecules that block viral RNA or protein synthesis. A second role of interferons is to stimulate and enhance the expression of MHC class I proteins on cell surfaces. This effect causes cells to better express viral proteins on their cell surface with MHC class I, thereby promoting CD8 T-cell activation in the adaptive immune response. Finally, interferons also activate natural killer (NK) cells, giving them the ability to attack and kill virus-infected cells.

Natural Killer Cells in Early Infection

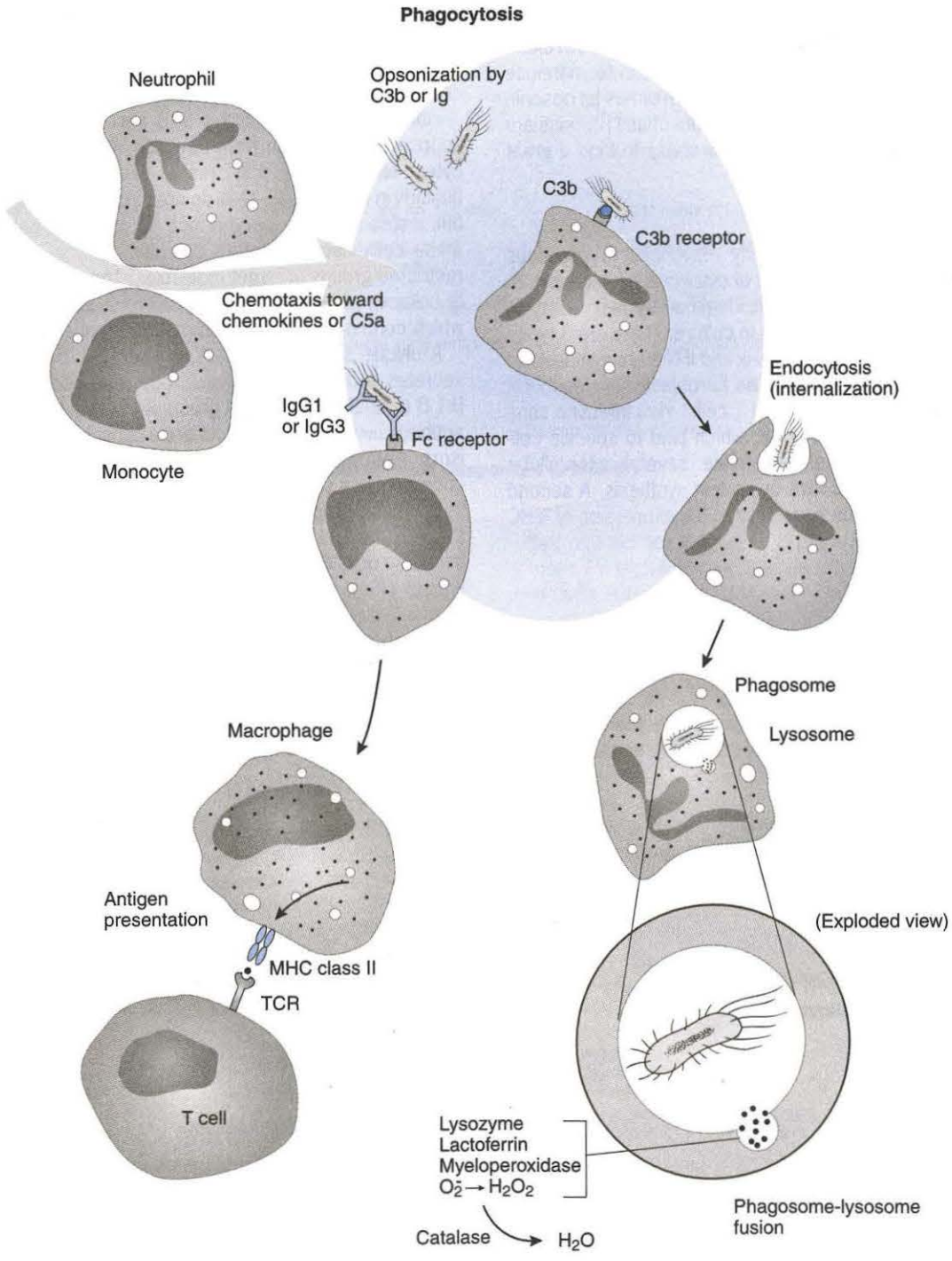
NK cells were first defined by their ability to kill selected tumor cells without having to be activated or immunized against the tumor cell. In this manner, NK cell cytotoxicity is not an adaptive immune response but can contribute to ongoing adaptive immunity. NK cells are also important in early host defenses against intracellular microorganisms (e.g., *Listeria monocytogenes*) and against viral infection. IFN-α, IFN-β, and IL-12 activate and enhance the killing properties of NK cells. Activated NK cells also produce IFN-γ, which itself has antiviral properties. NK receptors are thought to bind surface glycoproteins of virus- or bacteria-infected cells, although the exact mechanism of NK-mediated killing is uncertain. Virus-infected cells also often express lower levels of MHC class I molecules on their surface, thereby triggering NK-mediated recognition and lysis. Uninfected cells are spared from killing through NK cell surface receptors (killer inhibitory receptors, or KIRs) that bind MHC class I molecules. NK cells are also efficient at killing antibody-opsonized organisms by antibody-dependent cell-mediated cytotoxicity (ADCC).

Finally, 2 other cell types also perform nonspecific immune responses in protection from infection. A unique subset of T lymphocytes, termed *γδ T cells*, reside near epithelial cell surfaces particularly in the gut. γδ T cells do not circulate to other physiologic sites but instead provide surveillance to infecting pathogens. Although these cells have receptors on their surface, they appear to bind restricted groups of target molecules. The exact function of γδ T cells is unknown; however, they can also be activated to secrete IFN-γ, which contributes to innate immune responses as described earlier.

A unique subset of B cells, termed *B-1* B cells or *CD5* B cells, secretes a unique type of antibody response to infectious agents. B-1 B cells use a restricted group of variable region genes to secrete antibody binding the polysaccharide capsule of many infectious organisms. The antipolysaccharide responses from B-1 B cells do not undergo class switching or somatic mutation and do not require T-cell help. These properties make the B-1 response unique compared to the adaptive immune response found in normal B lymphocytes. B-1 B cells also do not develop the conventional memory immune responses typical of most B cells. The B-1 immune response occurs rapidly (within 2 days) after exposure to the pathogen. The antibody is primarily of IgM isotype and also has the capacity to bind other cross-reactive antigens. These properties account for its role in the innate and early immune response to bacterial infection.

For more information see Anderson, *IMS: Immunology*. Fence Creek, chapter 2.

10 Phagocytosis



Phagocytosis is an important mechanism of the innate immune system in the uptake, ingestion, and destruction of foreign antigens such as bacteria. Two forms of phagocytic mechanisms exist, *endocytosis* and *pinocytosis*. Endocytosis utilizes ATP energy metabolized in the cell as well as the synthesis of new membrane components and cytoplasmic proteins that orchestrate cell contraction and movement. Pinocytosis requires little cellular metabolism and is involved in the internalization of small solutes and fluids. The most important phagocytic cells of the immune system are *neutrophils*, *monocytes*, and *macrophages*. Neutrophils, also known as granulocytes or polymorphonuclear leukocytes, are found in highest concentrations in the peripheral circulation and are sensitive to chemotactic factors released at the site of infection. Monocytes are also circulating cells that develop into macrophages once they take up residence in peripheral tissues.

Active infection in tissues initiates the production of several important compounds that attract phagocytic cells (**Figure**). Endotoxin, also known as lipopolysaccharide (LPS), is a component of the outer membrane of gram-negative bacteria. LPS activates the alternative complement pathway leading to the production of C5a. C5a is a potent chemotactic factor for both neutrophils and macrophages toward the site of infection. C5a also enhances the adherence properties of neutrophils to vascular endothelium. Active inflammatory reactions at the site of infection and initiated by lymphokines such as TNF-α also promote chemoattraction and adherence of phagocytic cells. Once at the site, macrophages and other cells such as fibroblasts and keratinocytes release a host of chemokines including IL-8, MCP-1, MIP-1α, MIP-1β, and RANTES. These individual chemokines further attract T cells, neutrophils, and monocytes (see chapter on cytokines and chemokines for greater detail of these biological functions).

The mechanism of phagocytosis into the cell requires interaction with one of several surface receptors on the phagocyte. Uptake is facilitated by the *opsonization*, or coating of the particle to be ingested. The two most important opsonins are the C3b complement component and antibodies (IgG1 and IgG3) that are capable of binding Fc receptors on the surface of phagocytes (**Figure**). Opsonins are compounds that bind to the surface of the target antigen and also to the surface of the phagocytic cell. These compounds provide adhesion to the phagocytic cell and trigger the cellular synthesis and uptake of the foreign particle. The activation of complement by antibody complexed to the bacteria generates the cascade of activated complement products including C3b bound to the bacteria's surface. Receptors for C3b are found on the surface of phagocytes and facilitate the adhesion and uptake of the bacteria. In addition, antibody bound to bacterial surface also adheres to receptors for the Fc region of the Ig molecule. Bacteria that are opsonized in these ways are readily engulfed by phagocytic cells. Fc receptors on the surface of neutrophils and macrophages bind primarily to IgG1 and IgG3, and to a lesser extent, IgG4 and IgA2. Other proteins released at the site of infection, including fibronectin, which enhances adhesion between neutrophils and bacteria, and leukotrienes (LTB_4), which act as chemotactic factors, also enhance phagocytosis.

Phagocytosis initiates the folding of the outer membrane to engulf the particle to be ingested. The particle becomes completely surrounded by membrane and internalized into an intracellular compartment called a *phagosome*. Destruction of the engulfed particle is initiated by the fusion of the phagosome with lysosomes, the latter containing a number of hydrolytic enzymes. Neutrophils possess two types of lysosomal granules: azurophilic hydrolytic enzymes such as *lysozyme* and *myeloperoxidase*, and a second group that contains *lysozyme*, *alkaline phosphatase*, and *lactoferrin*. Lysozyme specifically attacks and digests bacterial cell wall compounds, causing their lysis. Other hydrolytic enzymes digest viral and bacterial proteins and enveloped membranes. Finally, lymphokines including IFN-γ and TNF-α stimulate phagocytic cells to produce nitric oxide synthases. Nitric oxide is toxic for many infectious agents including fungi, tumor cells, and some parasites.

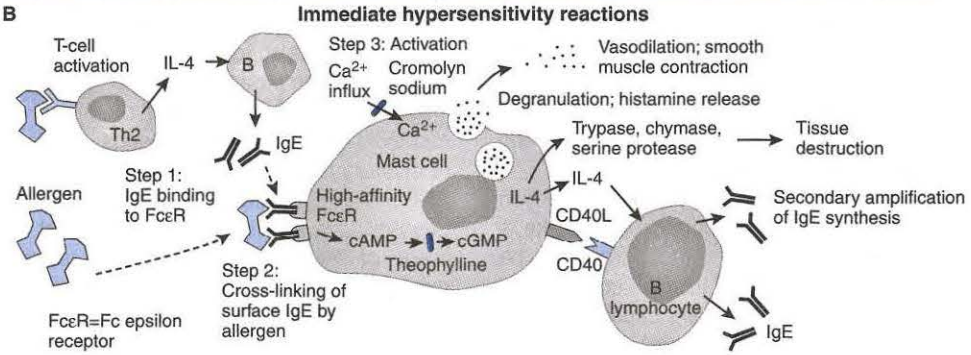
Toxic compounds are also produced inside phagocytes by the metabolism of oxygen compounds. Activated phagocytes undergo a respiratory burst, leading to the production of hydrogen peroxide (H_2O_2) that is released by the neutrophil and is toxic to bacteria. Superoxide anion is another product of oxygen metabolism found inside of lytic vesicles. Superoxide anions are very unstable and quickly broken down inside the cell. Similarly, H_2O_2 is neutralized inside the cell by the compound *catalase*. These mechanisms prevent the oxygen compounds from destroying the phagocyte itself. Patients with chronic granulomatous disease (CGD) have defects in the formation of oxygen compounds inside of neutrophils, accounting for the inability to clear bacterial infections. Lactoferrin is bacteriocidal by its binding to iron, which is required for bacterial survival.

11 Type I, Immediate Hypersensitivity (Allergy)

A

Common Allergens	Response	Clinical Expression
Drugs (penicillin), some foods (peanuts), venoms	Systemic anaphylaxis	Circulatory collapse; Hypotension, tracheal swelling Vascular permeability, edema Death
Foods: shellfish, eggs, dairy products (milk)	Food allergy	Diarrhea, vomiting Urticaria (hives) Pruritis (itching)
Allergy skin testing, insect bites (mosquito, bee stings)	Wheal-and-flare reaction	Vascular permeability, Rapid swelling, redness of skin
Pollen (ragweed), house dust, mite feces	Allergic rhinitis (hay fever)	Vascular permeability of nasal mucosa, runny nose
Pollens	Asthma	Bronchial inflammation, airway constriction, difficulty breathing

B



C

Macromolecules released from IgE-activated mast cells, basophils, and eosinophils	Biologic Activity
Histamine	Vascular permeability, smooth muscle contraction
Tryptase, chymase, serine proteases	Connective tissue digestion/remodeling
IL-4	Th2-cell stimulation, B-cell help for IgE synthesis
IL-8	Leukocyte chemotaxis
IL-5, GM-CSF	Granulocyte growth (eosinophils)
TNF-α	Local and systemic inflammation, promotes cytokine synthesis
Leukotrienes (C4 and D4)	Vascular permeability, smooth muscle contraction
Platelet-activating factor (PAF)	Platelet and granulocyte activation, leukocyte chemotaxis
Slow-reacting substance of anaphylaxis (SRS-A)	Vascular permeability, smooth muscle contraction
Neutrophil chemotactic factor of anaphylaxis (NCF-A)	Neutrophil chemotaxis
Eosinophil chemotactic factor of anaphylaxis	Eosinophil chemotaxis
Eosinophil collagenase	Connective tissue remodeling
Eosinophil peroxidase	Histamine release from mast cells
Heparin	Anticoagulation
Prostaglandins E_1 and E_2	Vasodilation, bronchodilation

Immediate, or *type I hypersensitivity* is more commonly known as *allergy* and is mediated by the IgE antibody subclass also known as reaginic antibody or "reagins" bound to a variety of environmental antigens (**Part A**). Approximately one-third of the population expresses IgE-mediated hypersensitivity to environmental antigens. This immune response is termed "immediate" because it relies on circulating preformed IgE antibodies that bind and initiate hypersensitivity within minutes of entry of the allergen into the individual. However, adaptive immunity utilizing IgE class antibodies evolved primarily for protection against parasitic worms, not for the generation of allergic responses. Like any adaptive immune response, allergy (also known as *atopy*)does not occur upon first exposure to the allergen but arises

after repeated antigenic stimulation and the development of helper T cells (Th2) and specific IgE antibodies. Symptoms of atopy can range from sneezing and nasal congestion typical of hay fever to systemic anaphylaxis or death, as can be observed in highly sensitive individuals in response to insect bites. The severity of type I hypersensitivity depends on the dose and route of entry of the allergen, with higher doses eliciting a greater response. In addition, a genetic predisposition to some forms of allergy (ragweed pollen) is linked to the expression of the MHC class II DR2 molecule.

Allergens most frequently enter through respiratory mucosal surfaces for exposure to the immune system. For example, pollen that is first inhaled may cross the respiratory epithelium and be engulfed by resident macrophages and dendritic cells for the presentation and activation of CD4 T cells. IL-4 production from activated Th2 cells drives IgE production by B lymphocytes. Continual reexposure to the pollen allergen amplifies the response until the threshold of circulating IgE elicits the degranulation of mast cells and subsequent release of vasoactive amines. As shown in **Part B**, preformed IgE binds high-affinity FcεRI receptors on the surfaces of mast cells, basophils, and activated eosinophils. The *cross-linking* of surface-bound IgE with the allergen causes degranulation of the mast cell or basophil and the subsequent release of vasoactive compounds such as histamines and a host of cytokines (IL-3, IL-4, IL-13, GM-CSF, TNF-α) and chemokines (**Part C**).

Most allergens are introduced through the respiratory system at low doses and easily traverse the mucosa by virtue of their small size (low molecular weight). Low-dose antigens favor IL-4–secreting Th2 responses important for switching IgM to IgE production by B cells. Th1 cytokines, such as IFN-γ, reduce allergic responses by promoting the production of other nonallergenic subclasses of Ig that do not bind FcεRI on mast cells. Moreover, activated mast cells can amplify IgE production by their ability to express CD40L and IL-4 in a manner related to activated Th2 cells. Surface CD40L binds to CD40 present on the surface of B lymphocytes. Both IL-4 and CD40-CD40L interactions promote IgE production.

Most Fc surface receptors only bind Ig when complexed to its antigen. FcεRI is an exception to this rule in its ability to bind free IgE molecules. This phenomenon allows atopic IgE-bound mast cells in the mucosa and subepithelial tissues to be poised for immediate activation and degranulation within seconds after exposure to the allergen. Thereafter, inflammatory responses (vasodilation and chemoattraction) draw basophils and eosinophils to the site, enhancing chronic morbidity and tissue pathology. Enzymes released by mast cell degranulation—trypase, chymase, and serine proteases—break down structural tissue proteins in perpetuating the allergic response.

Systemic anaphylaxis is a much more serious and potentially life-threatening expression of immediate hypersensitivity. Systemic anaphylaxis is a result of allergen exposure in a systemic fashion, either by introduction of antigen to the circulation, as in drug reaction (e.g., penicillin) and some insect bites, or by adsorption of large doses of antigen via the gastrointestinal mucosa. Systemic degranulation of mast cells causes a precipitous drop in blood pressure from the release of vasodilator compounds, the contraction of smooth muscle, and the swelling and constriction of airway passages that may lead to suffocation. The use of epinephrine can usually prevent the mortality associated with this extreme hypersensitivity response.

Allergic rhinitis, also known as hay fever, is a less severe immediate hypersensitivity response triggered by the introduction of respiratory allergens. Mast cells in the nasal tract epithelium degranulate upon antigen binding, creating histamine-induced swelling and typical "runny nose" exudates that may be enriched in eosinophils. In contrast, *asthma* is a more severe response to allergens caused by mast cells in the lower respiratory system. Breathing becomes difficult due to an influx of fluids and cells into the bronchial tract, usually requiring prompt antiinflammatory intervention. These responses may become severe, as chronic asthmatics have continuous resident populations of mast cells, eosinophils, and Th2 T cells within the lower bronchial epithelium.

Cutaneous hypersensitivity may be manifested by *eczema (atopic dermatitis)*, a chronic atopic response in infants, or other more immediate skin allergic responses found at any age.

The identification of common allergies is often performed by the injection of small amounts of individual allergens below the skin. The *wheal-and-flare skin reaction* caused by the presence of allergen-specific IgE, mast cell degranulation, and the dilation of blood vessels in the dermis causes redness, with a characteristic inflammation arising several hours later. The wheal-and-flare response is also the common allergic response to insect bites. Alternatively, allergen-specific serum IgE can be measured by enzyme-linked immunosorbent assay (ELISA) in the laboratory.

Treatment of Immediate Hypersensitivity

Besides avoidance of the source of the allergen if possible, treatment of hypersensitivity includes use of agents that interfere with the vasoactive effects of histamines (antihistamines such as diphenhydramine) or with the IgE receptor of mast cells, or immunomodulation of the Th2 response critical in the induction of IgE-secreting B cells. The latter method of immunomodulation, termed *desensitization* or *hyposensitization*, is performed by subcutaneous injection of gradually increasing amounts of a known allergen into the affected individual. The theory is to decrease the T-cell response from Th2 (secreting IL-4, IL-5, IL-6, and IL-10) to Th1 (secreting IFN-γ, IL-2, and TNF-α). This approach is designed to reduce the levels of atopic IgE and promote the synthesis of IgG class antibodies. Some specific antibodies, such as anti–insect venon IgE, can provide good protection to individuals.

Epinephrine is a rapid-acting agent that promotes the relaxation of smooth muscle and stimulates cardiac function. It is the most effective acute treatment of anaphylaxis. *Corticosteroids* are effective treatment for chronic inflammatory responses in allergic rhinitis or asthma. Steroids are thought to stabilize membrane structures, such as those enclosing intracellular granules to help prevent degranulation.

Several agents interfere with intracellular signaling events that are critical in mast cell activation and degranulation. These agents include cromolyn sodium, which blocks Ca^{2+} influx into activated cells, and theophylline, which inhibits phosphodiesterase and maintains intracellular cAMP levels.

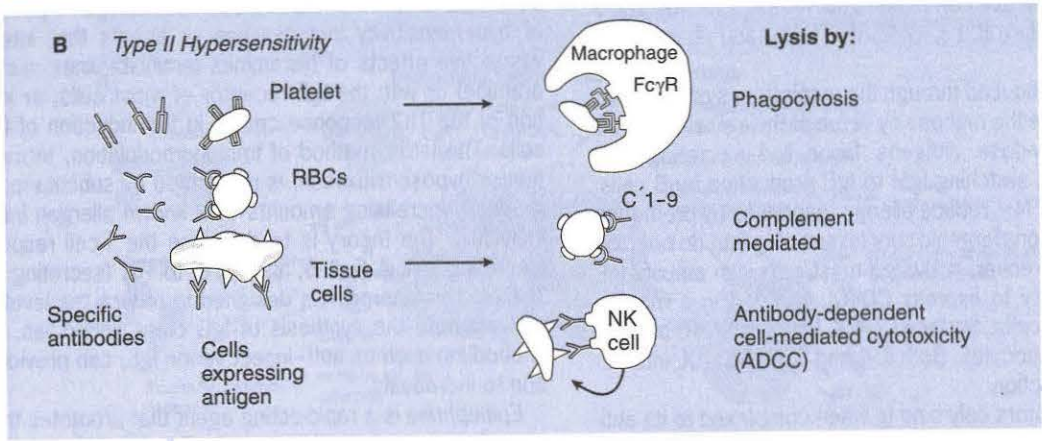
The response of immune cells is based in part on the dose and route of individual antigens. Minute quantities of antigen usually elicit no immunity while extremely high antigen doses can overwhelm lymphopoietic cells, causing a less-than-ideal response. Antigens administered subcutaneously and in the presence of adjuvant (or immunostimulatory bacterial products) provoke the strongest immunity while antigen given intravenously often induces systemic tolerance. Likewise, food antigens exposed to immune cells in the gut may elicit local immunity and also systemic unresponsiveness, accounting for tolerance to food antigens. Orally administered prototype antigens cause systemic immune tolerance in several laboratory models.

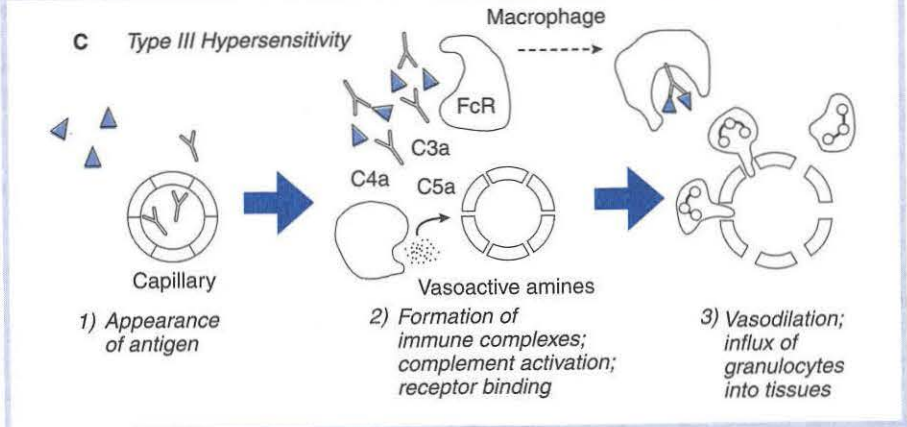
Allergies can be treated by the subcutaneous administration of low doses of the specific allergen over an extended period of time, so-called low-dose tolerance. The exact mechanism of this form of immune suppression is not clearly understood.

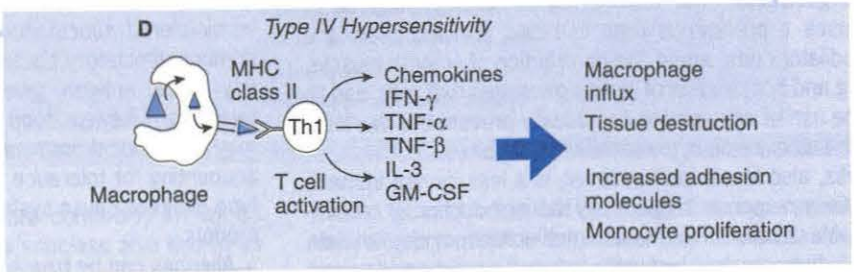
12 Type II, Type III, and Type IV Hypersensitivity Reactions

A Table 1

Type of Hypersensitivity	*Critical Immune Component*	*Mechanism*	*Common Clinical Syndromes*
Type I	IgE	Mast cell activation, degranulation	Allergies, allergic rhinitis asthma, systemic anaphylaxis
Type II	IgG (some IgM)	Lysis of antigen-bound cells	Hemolytic anemia, ITP, drug allergy (penicillin)
Type III	IgG	Deposition of immune complexes	Serum sickness, Arthus reaction
Type IV	Th1 cells	Macrophage activation, cytokine release, inflammation	Tuberculin skin testing, contact dermatitis
	Cytoxic T cells	Lysis of antigen-bound cells or tissues	Contact dermatitis







Three additional types of hypersensitivity are mediated by other components of the immune system, including IgG, immune complexes, and T lymphocytes (**Part A**). Although all constituents of the immune system evolved to protect the body from infectious agents, under some conditions these cells or macromolecules can also interact with innocuous antigens and lead to either acute or chronic hypersensitivity pathologies.

Type II Hypersensitivity

Type II hypersensitivity reactions are mediated by antibody bound to antigen on the surfaces of cells or tissues. Antigen may be naturally expressed on the cell surface or artificially bound, as in the attachment of certain drugs to cell surfaces. Drugs that mediate type II responses in some individuals include some antibiotics (penicillin), antihypertensive agents (methyldopa derivatives), and some drugs for the treatment of cardiac arrhythmia (quinidine). The drug must be capable of eliciting IgG or IgM class antibody responses (antidrug antibodies).

The end product of type II hypersensitivity is the lysis of the target cell. Lysis can be accomplished by: 1) complement-mediated lysis; 2) phagocytosis of opsonized (antibody-coated) cells via Fcγ receptors, with or without complement activation; 3) lysis by natural killer (NK) cells via antibody-dependent cell-mediated cytotoxicity (ADCC).

The most important type II reactions occur in the lysis of red blood cells (RBCs). These include transfusion reactions, Rh incompatibility, erythroblastosis fetalis, autoimmune hemolytic disease, and idiopathic thrombocytopenia purpura.

The most severe *transfusion reactions* arise due to ABO blood group incompatibility between the donor and recipient of RBCs. Immediate intravascular hemolysis of RBCs occurs because of the binding of preformed antibodies to the A or B antigen with subsequent complement-activated lysis. These reactions now occur only rarely, owing to the availability of high-quality ABO compatibility testing. Symptoms of severe transfusion reactions include acute renal failure accompanied by fever, chills, malaise, nausea, hypotension, and vomiting.

RBC lysis can also occur in the context of Rh surface antigens, which elicit strong antibody responses in individuals lacking the appropriate Rh_o (D) antigen. Antibodies elicited to the Rh antigens opsonize (coat) the RBC, which is then phagocytosed by macrophages or neutrophils. Complement-mediated lysis is typically not a mechanism of Rh incompatibility responses.

In addition, there are two types of *hemolytic anemia*, termed warm and cold. *Warm hemolytic anemia* is due to IgG class anti-Rh determinants with or without complement that react best with RBCs at 37°C. Phagocytosis is the primary mechanism of RBC depletion. In contrast, *cold hemolytic anemia* is caused by complement-fixing IgM antibodies that bind and agglutinate RBCs at 4°C. The IgM antibodies are specific for RBC antigen I and can arise in response to some infections such as with *Mycoplasma pneumonia*.

Erythroblastosis fetalis arises when an Rh-positive infant is born to an Rh-negative mother. It is common for small numbers of fetal RBCs to cross the placenta into the mother's circulation. This occurs particularly during birth when infant cord blood cells find their way into the mother to elicit anti-Rh antibodies. The first child born to an Rh-incompatible mother is not at risk for the clinical sequelae, since the mother has only become sensitized (or immunized) to the Rh antigens. During a second pregnancy, maternal anti-Rh antibodies cross the placental barrier to cause lysis of fetal RBCs. The infant experiences anemia and jaundice with splenomegaly. Only the most severely affected infants require transfusions as treatment for this syndrome. The induction of antibodies can be blocked by treatment of the mother with anti-Rh IgG antibodies, termed *RhoGAM*, at approximately 25 weeks of gestation and again at parturition. These antibodies bind and eliminate Rh-positive fetal cells from the maternal circulation before an immune response can be mounted. The mother must be treated during every pregnancy because tolerance to the Rh antigen is not established by this therapy.

Idiopathic thrombocytopenia purpura is a type II response due to the binding of platelets by antiplatelet IgG antibodies. The outcome is a host of bleeding disorders due to the inability to properly form clots. Antibodies against other lymphoid cells (e.g., T lymphocytes) can arise in autoimmune syndromes such as systemic lupus erythematosus (SLE).

Type III Hypersensitivity

The deposition of antigen-antibody *immune complexes* is the pathologic mechanism of type III hypersensitivity. This response requires the presence of soluble (not cell-bound) antigen able to provoke complement-fixing IgG antibody. As illustrated in **Part C**, Ig–antigen complexes form lattices that deposit in the walls of blood vessels or in tissue spaces, depending on the size of the complex. The classic Type III immune response is the *Arthus reaction*. The Arthus reaction has been historically demonstrated by the injection of antigen under the skin of an individual (or laboratory animal) that possesses preformed IgG antibodies to the antigen. Complement-activating immune complexes form at the site and bind Fc receptors on local leukocytes. Vascular permeability is enhanced, causing erythema and the influx of neutrophils and fluids to the site, all responses typical of inflammation.

Serum sickness is another classic type III hypersensitivity response, described by the use of serum antibodies from an immune foreign species injected into humans. The precipitation of immune complexes into tissues leads to the characteristic symptoms of fever, vasculitis, arthritis, and occasionally nephritis. *Farmer's lung* develops from the IgG immune complexes that form with high levels of respiratory antigens such as crop dust or mold spores. The deposition of complexes into the alveoli elicits inflammation and an inadequate ability to perfuse oxygen into the blood. Treatment of type III inflammation is with aspirin, corticosteroids, or antihistamines. More severe responses are treated with immunosuppressive drugs (cyclophosphamide or azathioprine) or by plasmapheresis for the removal of circulatory immune complexes.

Type IV Hypersensitivity

Type IV hypersensitivity, also known as *delayed-type hypersensitivity (DTH)*, is mediated by antigen-specific T lymphocytes, either Th1-type (CD4+) cells or cytotoxic (CD8+) T cells (**Part D**). It is termed "delayed" because the response requires 24–48 hours to develop after contact with antigen, in contrast to type I responses, which arise within minutes. DTH responses take more time to develop because pathology first requires the activation of T cells and the induction of inflammatory cytokines followed by their biologic responses (i.e., vasodilation and the attraction of cells). Small metal ions (nickel or chromate) and some haptens (dinitrofluorobenzene) can cause *contact hypersensitivity* by attaching themselves to proteins in the host. The modified host proteins are then presented to antigen-specific T cells for activation.

The response to *tuberculin skin testing* is the prototype DTH response and is a diagnostic tool to determine whether an individual has been infected with *Mycobacterium tuberculosis*. A small quantity of antigen extract is injected under the skin, where macrophages present antigen in the context of MHC class II to Th1 cells. Activation of the antigen-specific Th1 cells causes release of inflammatory lymphokines and chemokines, eliciting vascular permeability and the entry of fluids and cells, primarily macrophages, to the site. Erythema and induration at the site of injection indicate exposure to the tuberculosis organism. The cytokines critical to this response include IFN-γ, TNF-α, TNF-β, IL-3, and GM-CSF (see Chapter 8).

Cytotoxic CD8+ T cells are a second mediator of DTH responses. Some antigenic compounds such as plant toxins (e.g., poison ivy) can cross the lipid bilayer membrane of antigen-presenting cells to bind cytosolic proteins and enter the class I presentation pathway. Activated CD8 T cells release IFN-γ and other inflammatory mediators as well as cause direct tissue damage via cell-mediated cytolysis.

13A Immunosuppression/Immunomodulation

A

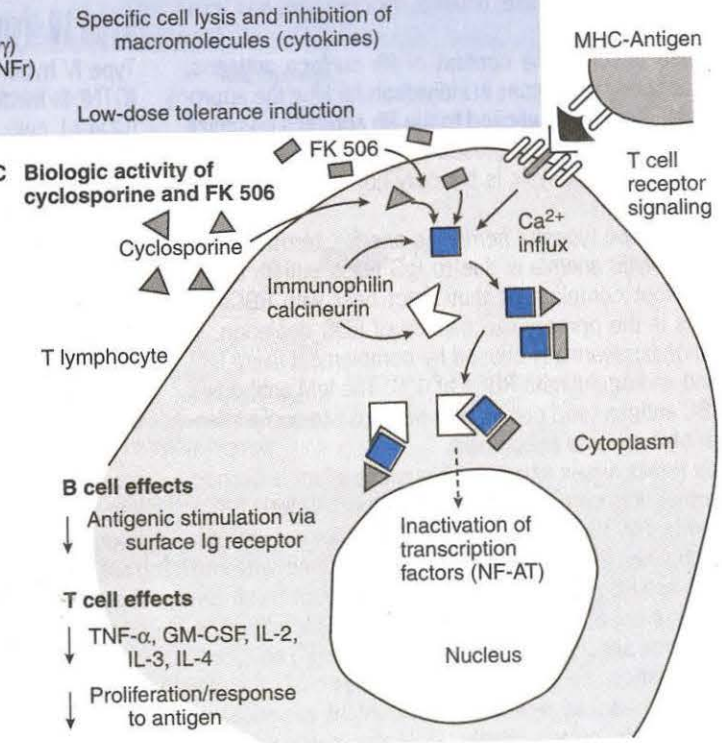
Nonspecific immunosuppression	Biologic Effects
Antiinflammatory drugs	
Corticosteroids (prednisone)	Inhibitors of cytokines, nitric oxide synthase, cyclooxygenase, and adhesion molecules
Nonsteroidal antiinflammatory drugs (NSAIDs)	
Aspirin (acetylsalicylic acid)	Inhibitors of cyclooxygenase, prostaglandin synthesis
Ibuprofen (phenylacetic acid)	
Indomethacin (indoleacetic acid)	
DNA metabolism inhibitors	
Cyclophosphamide	Alkylating agent for DNA
Chlorambucil	Alkylating agent for DNA
Methotrexate	Folic acid analogue
Azathioprine	Purine analogue
Inhibitors of T cell signaling	
Cyclosporine	Immunophilin/calcineurin signaling inhibitor
FK 506 (tacrolimus)	Immunophilin/calcineurin signaling inhibitor
Rapamycin (sirolimus)	IL-2 receptor signaling inhibitor

Specific immunosuppression	Biologic Effects
Monoclonal antibodies to	
T cells	Specific cell lysis and inhibition of macromolecules (cytokines)
Cytokines (IL-1, IL-2, TNF-α, IFN-γ)	
Cytokine receptors (IL-1r, IL-2r, TNFr)	
Adhesion molecules (ICAM)	
Specific allergens	Low-dose tolerance induction

B *Specific Biologic Effects of Corticosteroids*

1. Decreased interleukin synthesis including IL-1, IL-4, IL-8, TNF-α, and GM-CSF. Proinflammatory cytokines are inhibited, leading to decreased vasodilation and cellular influx.
2. Decreased cyclooxygenase, decreased phospholipase A synthesis, causing a decrease in prostaglandin and leukotriene synthesis.
3. Decreased production of nitric oxide synthase and nitric oxide. Nitric oxide is critical in the killing of pathogens by phagocytes.
4. Decreased synthesis of adhesion molecules, resulting in a decrease in lymphocyte migration to sites of inflammation.

C Biologic activity of cyclosporine and FK 506



Since the immune system evolved for the surveillance and clearance of infectious agents, it may be hard to understand why suppression of normal immune responses would be beneficial to the host. Immunosuppression can come in many forms, including natural genetic alterations, surgical intervention, and as a result of exposure to radiation, synthetic drugs, and natural products of the immune system. Immunosuppressive conditions can also be mediated by infectious agents (such as HIV), by malignancy, or by malnutrition. The three most obvious conditions in which suppressed immune responses are beneficial are 1) the survival of transplanted tissues and allografts, 2) the suppression of allergic responses, and 3) the suppression of spontaneous autoimmune diseases.

As indicated in other chapters, the immune system has many self-regulatory mechanisms in place including suppressor T cells, suppressive lymphokines, and the immune regulation of various cell surface molecules such as B7 co-stimulatory molecules and MHC class I and class II surface proteins. Other factors such as stress, which induces the release of corticosteroids as well as other naturally arising hormones including ACTH, prolactin, growth hormone, and testosterone, have immunomodulating effects. Antiidiotypic antibody networks may help regulate ongoing humoral immune responses. Products of the sympathetic nervous system such as norepinephrine, endorphins, and enkephalins can be bound by specific receptors on lymphocytes and macrophages.

As illustrated in animal models, surgical removal of primary and secondary lymphoid organs often has dramatic suppressive effects on immune responses. Although all immune cells originate as pluripotent bone marrow cells, differentiation into specific cell subsets occurs in peripheral organs. Removal of the thymus in the neonatal period totally ablates T-cell development. Similarly, surgical removal of the bursa of Fabricius, an organ for B-cell development in avian species, eliminates B-cell development. In contrast, removal of these organs from adults may cause little or no immune system effects because effector and memory lymphocytes have already seeded many tissues including peripheral lymph nodes. Removal of lymph nodes has little or no effect on immunity, as these tissues are diffuse in location and are connected by a vast network of lymphatic channels.

Immunosuppression by Drugs and Natural Biologic Macromolecules

The principal role of chemicals and biologic suppression is to control allergic responses (hypersensitivity), autoimmune disease, transplantation rejection responses, and cancer. Drug therapy often does not have specific immunologic targets for activity but rather provides generalized immunosuppression. Chemical agents are best able to interfere with primary immune responses to a particular antigen (first exposure to the antigen) instead of modifying secondary immune responses involving memory B and T lymphocytes. Memory immune responses have proved difficult to modulate with drugs or biologic agents. Drug therapies can be found from three principal groupings: cytotoxic drugs (azathioprine, cyclophosphamide), corticosteroid antiinflammatory drugs (prednisone), and products of bacteria or fungi (cyclosporine, FK 506, and rapamycin (**Part A**). Biologic immunosuppressants are primarily found as antibodies administered to specific surface molecules or proteins that bind and neutralize cytokines or their receptors.

Corticosteroids

Corticosteroids are among the most widely used antiinflammatory and immunosuppressive drugs for a variety of clinical syndromes including autoimmunity, allergy, and treatment of organ transplantation (**Part B**). Synthetic corticosteroids have wide-ranging biologic effects, as virtually every cell has glucocorticoid receptors that modulate specific transcription of genes at the molecular level. The antiinflammatory effects of corticosteroids are a result of decreased proinflammatory cytokine synthesis and a decrease of the expression of adhesion molecules on leukocytes. The cytokines downregulated by steroids include IL-1, TNF-α, GM-CSF, IL-3, IL-4, IL-5, and IL-8. As described in the chapter on cytokines, IL-8 and TNF-α enhance the expression of vascular adhesion molecules, E-selectin, P-selectin, and ICAM. Local tissue increases in vascular permeability caused by TNF-α are blocked with the use of corticosteroids. The outcome of these interactions is a decrease in the influx of cells and fluids to sites of inflammation. In addition, steroids inhibit nitric oxide synthase, an important molecule in generating nitric oxide in phagocytic cells, which kills intracellular organisms. Corticosteroids decrease prostaglandin and leukotriene levels as a result of reduced phospholipase A and cyclooxygenase metabolism in the cell. Overall, corticosteroid treatment reduces the interaction of lymphocytes with antigen by inhibiting cellular chemotaxis and by downregulating proinflammatory cytokine responses. Corticosteroids are widely used inhibitors of inflammatory responses typical of autoimmunity such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).

A host of nonsteroidal antiinflammatory drugs (NSAIDs) exist for the treatment of SLE and RA. NSAIDs primarily act by inhibition of the cyclooxygenase pathway, which causes downstream inhibition of arachidonic acid metabolism into proinflammatory prostaglandins. Aspirin is the most commonly used and well-described NSAID. NSAIDs typically have rapid effects mediated by their binding to plasma proteins and access to sites of inflammation. Other effects include inhibition of platelet aggregation (anticoagulant activity), suppression of bradykinins, and inhibition of phagocytic activity by macrophages and neutrophils.

Cytotoxic Drugs

Cytotoxic drugs act by inhibiting DNA synthesis, a process most apparent in rapidly dividing cells. Indeed, these drugs were first derived for the treatment of cancer cells, although the observation that lymphocytes are rapidly dividing cells provided a second effect of these agents as powerful immunosuppressants. Susceptibility to cytotoxic agents is also found in intestinal epithelium and in hair growth. Side effects of cytotoxic drugs include anemia, thrombocytopenia, and leukopenia. These agents are commonly used in combination with other immunosuppressants such as corticosteroids that require careful monitoring of clinical responses. Bone marrow suppression and lymphoproliferative malignancies can be a consequence of long-term use of azathioprine.

Cytotoxic drugs act by inhibiting purine metabolism in the cell, adenine and guanine, two components of DNA synthesis. These drugs either block intermediate synthesis of purines or cross-link DNA, both with the result of blocking DNA synthesis. Cyclophosphamide and chlorambucil are in the family of alkylating agents that cross-link DNA and often are more effective in blocking B-cell function than T-cell responses. This makes cyclophosphamide an appropriate treatment for autoimmune diseases such as RA and SLE.

Methotrexate is another class of DNA synthesis inhibitor. Methotrexate is a synthetic analogue of folic acid and aminopterin (compounds essential for DNA synthesis) that inhibits dihydrofolate reductase activity.

13B Immunosuppression/Immunomodulation

A

Nonspecific immunosuppression	Biologic Effects
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Ibuprofen (phenylacetic acid)	
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DNA metabolism inhibitors	
Cyclophosphamide	Alkylating agent for DNA
Chlorambucil	Alkylating agent for DNA
Methotrexate	Folic acid analogue
Azathioprine	Purine analogue
Inhibitors of T cell signaling	
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Specific immunosuppression	Biologic Effects
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T cells	Specific cell lysis and inhibition of macromolecules (cytokines)
Cytokines (IL-1, IL-2, TNF-α, IFN-γ)	
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Adhesion molecules (ICAM)	
Specific allergens	Low-dose tolerance induction

B *Specific Biologic Effects of Corticosteroids*

1. Decreased interleukin synthesis including IL-1, IL-4, IL-8, TNF-α, and GM-CSF. Proinflammatory cytokines are inhibited, leading to decreased vasodilation and cellular influx.
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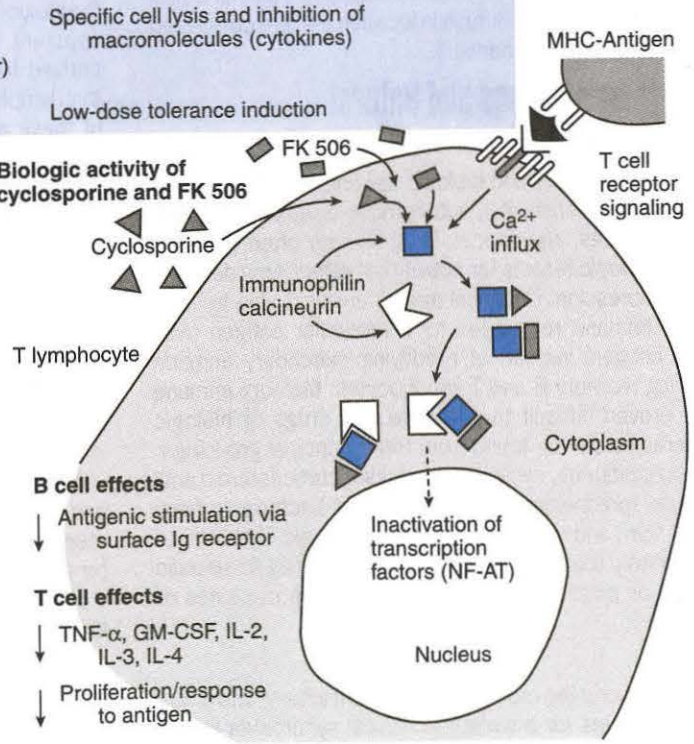
C Biologic activity of cyclosporine and FK 506



Immunosuppression by Microbial Products

Two principal immunosuppressive agents now commonly used in organ transplantation are cyclosporine, purified from fungus, and FK 506, derived from a filamentous bacteria, *Streptomyces* (**Part C**). These compounds have much less toxicity compared to the agents that interfere with purine metabolism and act by binding to intracellular signaling molecules, immunophilins, which are important for lymphocyte proliferation. Both cyclosporine and FK 506 inhibit the expression of a number of T lymphocyte–derived cytokines, including IL-2, IL-3, IL-4, TNF-α, and GM-CSF. Since IL-2 is an autocrine growth factor for T cells, its absence will significantly block T-cell responses to antigenic stimulation. The inhibition of IL-4 interferes with Th2 T-cell development as well as inhibits B-cell proliferation following stimulation of surface Ig receptors by antigen.

High doses of these drugs are typically used early in organ transplantation to prevent acute graft rejection. As tissues become engrafted over time, maintenance doses can be reduced to prevent chronic organ rejection. These drugs are particularly effective in inhibiting T cell–mediated autoimmunity or graft rejection. Either cyclosporine or FK 506 complexed to immunophilin interfere with calcineurin-mediated signal transduction. These interactions block activation of a nuclear transcription factor, NF-AT, which transcribes the IL-2 gene. Another drug in this family, rapamycin, is also derived from *Streptomyces* and binds to intracellular immunophilins. However, this drug blocks the signal transduction pathway mediated by the IL-2 receptor from the surface of the T lymphocyte.

Immunosuppression by Specific Antibodies

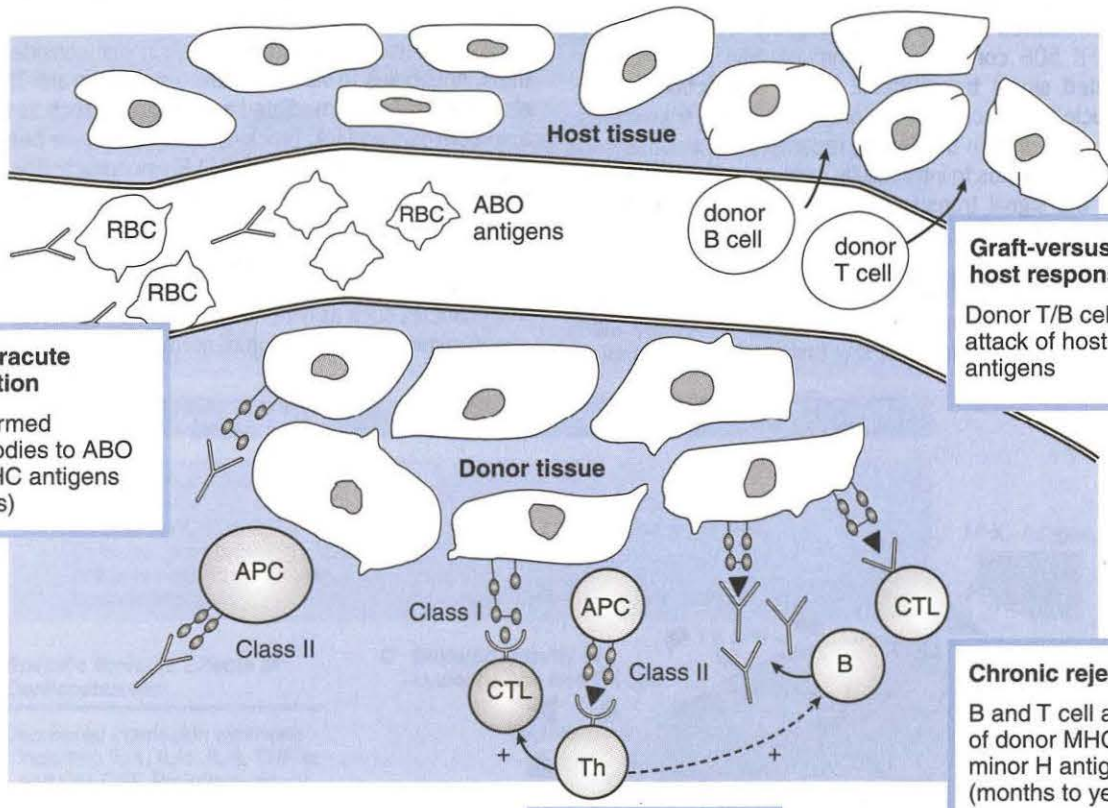
Antibodies are desirable as immunosuppressive agents for their high specificity to cell subsets and for their low toxicity. Historically, anti-lymphocyte antibodies derived from human Ig–immunized horses have been successful in blocking acute graft rejection. However, this therapy is not specific to lymphocyte subsets but instead eliminates all lymphocytes by complement-mediated lysis. The use of horse serum as a source of polyclonal antibodies often leads to serum sickness caused by immune complex formation when antihorse immunity develops in the human host.

More specific therapies have been initiated with monoclonal antibodies (antibodies of single specificity) developed in mice. Molecular biology has allowed for the purification of antigen-binding CDRs of the genes for monoclonal antibodies, which are then inserted into human framework genes for Ig. These "humanized" monoclonal antibodies elicit little or no immune responses that would cause immune complex formation and subsequent serum sickness. This technology has the benefit of offering precise specificity for individual cell subsets or soluble factors such as cytokines. Antibodies to the B7 co-stimulatory molecules have been utilized to block T-cell activation events that occur with B7-CD28 interactions. Anti-B7 antibodies are effective in inhibiting animal models of disease such as autoimmune diabetes and multiple sclerosis, and are presently under consideration for human trials. Antibodies to various cytokines such as anti-TNF-α and anti-IL-2 also prevent T cell–mediated autoimmunity such as that found in multiple sclerosis and RA. Blocking antibodies have been used for years in preventing red blood cell (RBC) Rh incompatibility (erythroblastosis fetalis). Antibodies to the Rh factors bind to fetal RBCs, causing their opsonization and gradual depletion.

Immunosuppression also has been observed in a variety of immunodeficiency syndromes, as described in another chapter. Finally, malignancies such as lymphomas may also be immunosuppressive by overwhelming the population of normal immunoreactive lymphocytes.

14A Transplantation

A **Immune-mediated Transplantation Rejection**



Hyperacute rejection

Preformed antibodies to ABO or MHC antigens (hours)

Graft-versus-host response

Donor T/B cell attack of host MHC antigens

Chronic rejection

B and T cell attack of donor MHC or minor H antigens (months to years)

Acute rejection

T cell activation to donor MHC or tissue antigens (10-30 days)

APC = antigen-presenting cell
CTL = cytotoxic (CD8) T cell
Y = antibody

Tissue transplantation has become a common procedure in many situations of organ failure. The increased frequency of organ transplantation and the high success rates of these operations are in part due to our enhanced knowledge of immune reactions against foreign tissues. The most common cause of organ rejection is from adaptive T-cell immune responses directed against *HLA (human leukocyte antigen)* proteins on the surfaces of transplanted tissue. The *major histocompatibility complex (MHC)* was so named because of its recognized importance in graft rejection. After the importance of MHC molecules became apparent in transplantation, greater attention was made to match donor and recipient MHC proteins. However, even under ideal situations donor and recipient tissues rarely have completely matched MHC antigens. Ideal donor-recipient pairs come from identical twin siblings; nonidentical siblings are the next best choice for organ transplantation. The inheritance of MHC genotypes predicts that 1 in 4 siblings will be identical at the MHC genes. Unfortunately, *minor histocompatibility antigens* (non-MHC proteins, also called minor H antigens) can also result in chronic tissue rejection mediated by immune responses.

Transplantation of tissues is defined by the origins of donor versus recipient. *Autograft* is the tissue transplanted within sites on the same individual. *Syngeneic graft* is tissue transplanted between genetically identical individuals. However, tissue transplantation most frequently occurs between genetically unrelated individuals; these tissues are termed *allografts*. *Xenograft* (or xenogeneic graft) is the term used to describe tissues transplanted between 2 different species (i.e., between animals and humans).

Success of tissue transplantation is apparent by the more than 30,000 heart transplantations performed since 1983, with a survival rate greater than 50% after 10 years. An even greater number of organ transplantations have been performed with kidney tissue, whereas bone marrow transplantation is a common therapy for a number of clinical syndromes including malignancies and immune deficiency disorders.

Histocompatibility Testing

The purpose of histocompatibility testing is to identify specific HLA class I and class II antigens from a graft recipient and a potential tissue donor. Histocompatibility testing is performed by two principal methods, the *lymphocytoxicity* test and the *mixed lymphocyte reaction (MLR)*. Lymphocytotoxicity utilizes antibodies to either class I or class II MHC antigens. Antibodies are available to most MHC antigens derived from patients who have received multiple transfusions or from multiparous women. In the latter case, women who have given birth to several children of the same father develop immunity to the father's MHC antigens, which transiently cross the placenta into the mother's circulation during birth. The mother generates antibody immunity to the HLA antigens, which are boosted upon subsequent births. A panel of sera specific for all of the MHC alleles is then assembled for serotyping the donor alleles.

For the cytotoxicity test, test lymphocytes are combined with the panel of antisera against HLA proteins. Upon the addition of complement, the lymphocytes are lysed only in samples in which the appropriate antibody-HLA complex has formed. The lymphocyte is lysed by conventional complement activation and cell death is identified by staining with vital dyes (trypan blue or eosin). Lysis by a specific antisera indicates the specific class I and class II antigens in the individual.

MLR is a common method employed to identify the potential compatibility between organ recipient and donor. The test is based on the proliferation of lymphocytes that naturally occurs when HLA incompatibility proteins on cells are mixed together. The interaction causes small resting lymphocytes to become activated into larger blast cells. The test is performed by mixing the test lymphocytes with a panel of X-irradiated donor lymphocytes. Irradiation causes the donor lymphocytes to become inert and not proliferate, while maintaining the ability to present HLA antigen to the test cells. Any observed proliferation is an indication that the recipient cells are incompatible with the donor cells.

Rejection Immune Responses

Three types of immune-mediated rejection reactions (**Part A**) can occur: 1) *hyperacute rejection*, 2) *acute rejection* (sometimes known as *accelerated rejection*), and 3) *chronic rejection* (**Figure**).

Hyperacute rejection occurs within minutes of tissue transplantation and is mediated by *preformed antibodies* to the donor tissue. Antibodies immediately bind the vasculature of the tissue transplant, causing activation of complement proteins and massive blood clotting. The response leads to obstruction of blood vessels feeding the graft, resulting in immediate death of the tissue due to lack of profusion. Historically, hyperacute rejection occurred most frequently because of mismatched ABO blood group antigens between donor and recipient. At the present time, hyperacute rejection rarely occurs, owing to the improved methods of HLA typing. Hyperacute rejection can occur when xenografts are transplanted from animal donor tissues into humans. For example, pigs have been utilized as a source of xenograft tissues because their organ size and function are similar to those in humans. Unfortunately, humans have several preformed antibodies to foreign species' tissues that mediate hyperacute antibody rejection as well as T-cell acute rejection (see below).

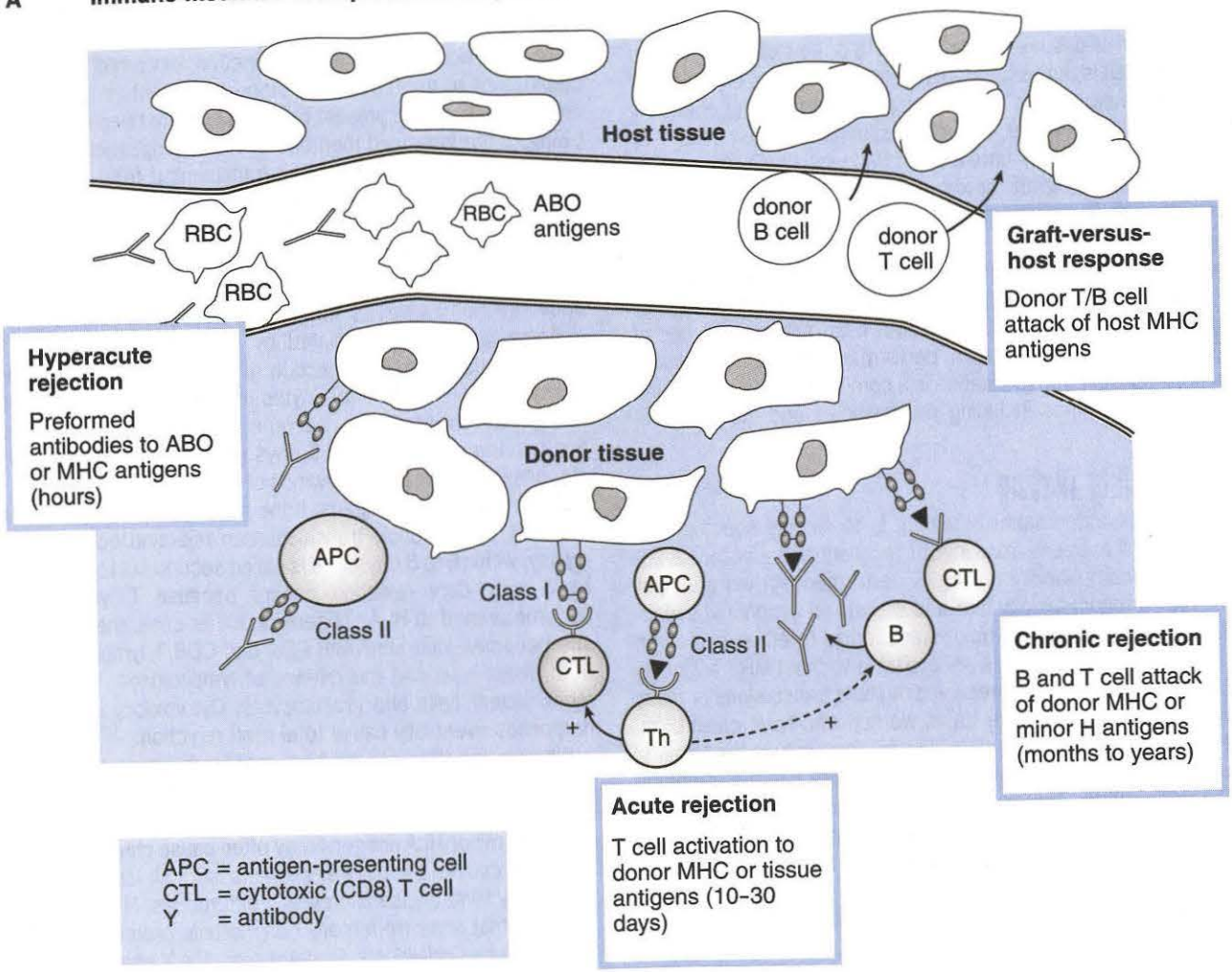
Acute rejection is mediated by *T lymphocytes* specific for the engrafted tissue. Acute rejection occurs within 10 to 30 days after transplantation, as T lymphocytes require time to be presented with foreign HLA antigen and to become activated. T cell–mediated rejection occurring from 11 to 15 days is termed *first-set rejection*. This response describes the activation of T lymphocytes to newly exposed transplanted tissue antigens. If the recipient receives a second graft from the same donor, transplantation rejection occurs much more rapidly, within 6 to 8 days, and is called *second-set rejection*. The more rapid secondary rejection occurs because T lymphocytes have become primed to HLA antigens. In either case, the transplanted tissue becomes infiltrated with CD4 and CD8 T lymphocytes, causing both direct lysis and the release of lymphokines, further attracting mononuclear cells and granulocytes. Cytotoxicity and inflammatory responses eventually cause total graft rejection.

Chronic rejection occurs from months to years after tissue transplantation and is mediated by both antibody and cellular immunity. Although major MHC antigens may be shared between donor and recipient, minor HLA antigens may often cause chronic graft rejection. Rejection caused by minor H antigens is much less severe than that caused by MHC class I and class II differences. Minor H antigens are peptides that arise from many polymorphic proteins within cells that differ between individuals. Proteins from the Y chromosome of males comprise one group of minor H antigens to which transplants into females cause chronic rejection reactions. Minor H antigen rejection responses typically can be controlled by immunosuppressive drugs.

Alloreactivity, the attack of mismatched MHC antigens by T lymphocytes, occurs when foreign MHC class I or class II antigens carry peptides that stimulate the host T-cell responses. Alloreactivity reflects the innate ability of T lymphocytes to respond to foreign MHC molecules, a reaction that should be identified by careful MLR testing.

14B Transplantation

A **Immune-mediated Transplantation Rejection**



Immunosuppressive Therapy for Transplantation

Virtually all recipients of allografts or transplants require long-term immunosuppression to decrease the chances of rejection. Occasionally, suppression may not be performed when grafts are exchanged between identical twins. Several immunosuppressive agents are utilized for posttransplantation therapy (for details, see chapter on immunosuppression).

Cyclosporine is a common drug of choice in transplant recipients. It is an agent purified from fungus and acts by interfering with DNA metabolism in lymphocytes. A related immunosuppressive agent, *FK 506*, also is purified from fungus and interferes with purine metabolism and subsequent DNA synthesis. Both cyclosporine and FK 506 bind to cyclophilin and interfere with lymphocyte signaling. The inability to signal lymphocytes causes a decrease in nuclear transcription factors, NF-AT, important in gene transcription in the nucleus. Both drugs interfere with the synthesis of cytokines including IL-2, IL-3, IL-4, TNF-α, and GM-CSF. IL-2 is an autocrine growth factor for T lymphocytes and its absence significantly downregulates T-cell immunity. Blocking IFN-γ synthesis is important, as this cytokine activates macrophages that are important components in rejection reactions. A third drug in this family, rapamycin, also impairs signal transduction through the IL-2 receptor of T lymphocytes. These drugs are typically utilized in high doses early in graft transplantation to prevent immediate graft rejection. Over time, doses are reduced to prevent chronic graft rejection. Two other alkylating agents, *azathioprine* and *cyclophosphamide*, interfere with DNA and RNA synthesis. Lymphocytes are particularly sensitive to these agents, resulting in immunosuppression.

Corticosteroids are often used in graft transplantation as maintenance therapy and in higher doses at times when more severe rejection reactions are apparent. Corticosteroids have a wide range of biologic effects causing the downregulation of many cytokines (IL-1, -3, -4, -5, -8, TNF-α, and GM-CSF). Corticosteroids also decrease levels of vascular adhesion molecules, *selectins* and *ICAM*, which are important for lymphocytes to infiltrate the engrafted tissue. Overall, corticosteroids reduce inflammatory responses, block the migration of cells into the graft, and reduce immunostimulating cytokines.

Some sites in the body have been termed "immunologically privileged." Tissues at these anatomic locations are not rejected, owing to the low concentration of MHC antigens. The cornea is the most well-known privileged site; others include cartilage, tendon, and major blood vessels. These tissues are most easily transplanted without great risk of rejection. Other sites including the brain (owing to the blood-brain barrier) and the anterior chamber within the eye are also privileged because of a lack of lymphatic vessels.

Graft-Versus-Host Disease

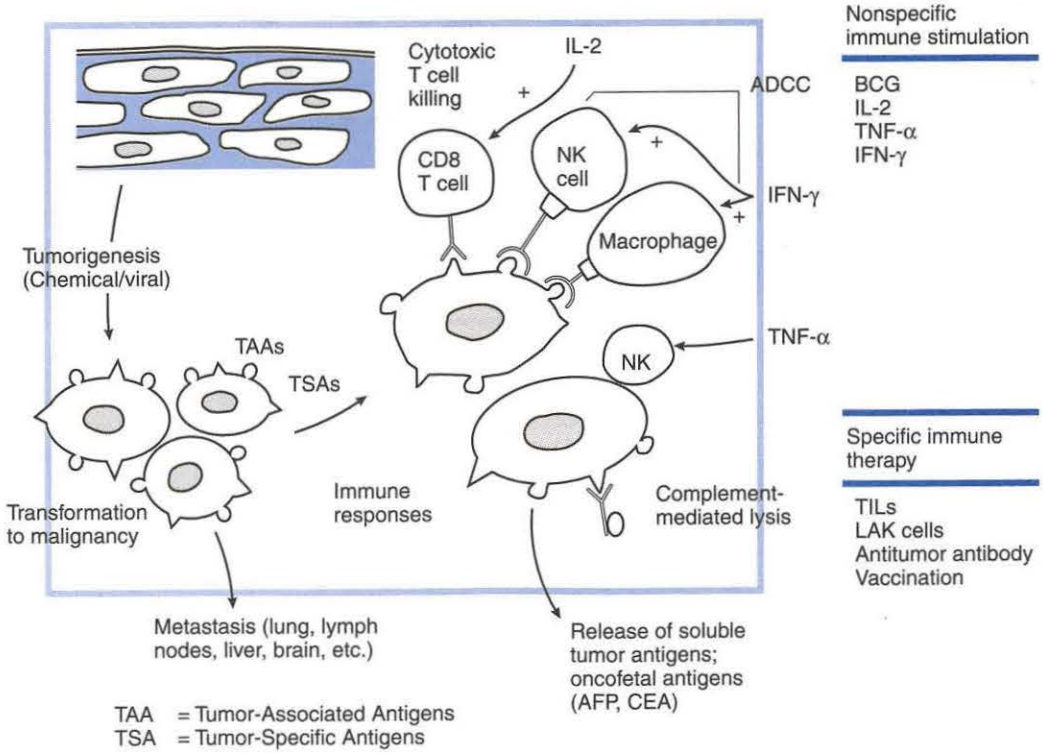
Graft-versus-host (GVH) disease is important in considerations for bone marrow transplantation. This immune response occurs when engrafted bone marrow attacks the recipient's tissues. The recipient is at risk because total-body irradiation wipes out the recipient's immune system prior to transplantation. The engrafted bone marrow becomes activated by the recipient's MHC antigens and initiates an attack. Close histocompatibility matching between donor and recipient and the removal of T cells from bone marrow prior to transplantation reduce the risk of GVH disease. GVH disease can also be treated with anti-T-cell antibodies, which lyse donor T cells by complement activation.

15 Tumor Immunology

A

Antigens Found in Human Malignancy	Malignancy
Oncofetal antigens	
α-fetoprotein (AFP)	Liver, testicular, ovarian, pancreatic cancers
Carcinoembryonic antigen (CEA)	Colon, pancreatic cancers
Viral associations with malignancy	
Epstein-Barr virus	Burkitt's lymphoma, nasopharyngeal carcinoma
Papillomavirus	Cervical carcinoma
Hepatitis B virus	Liver cancer
HIV	Kaposi's sarcoma, non-Hodgkin's lymphoma, brain lymphoma, Burkitt's lymphoma
Other associated proteins	
Myeloma proteins (immunoglobulins)	Multiple myeloma
Prostate-specific antigen (PSA)	Prostate cancer
Prostatic acid phosphatase	Prostate cancer
Common acute lymphoblastic leukemia antigen (CALLA)	B cell leukemia
Terminal deoxytransferase (TdT)	Leukemia, lymphomas

B



TAA = Tumor-Associated Antigens
TSA = Tumor-Specific Antigens

The immune system functions in the surveillance of neoplastic cells as they arise in the body. This phenomenon is best illustrated by the higher frequency of cancers in immunocompromised individuals (e.g., Kaposi's sarcoma in AIDS patients). Lymphomas more frequently arise in the immunosuppressed as does acute myelogenous leukemia in patients with Wiskott-Aldrich syndrome, an immune deficiency disorder. Overall, neoplasms arise most frequently in neonatal infants or the elderly when immune system functions may be failing. *Benign neoplasms* usually do not cause death, are typically slow growing, and are restricted to the site in which they arise. In contrast, *malignant neoplasms* spread to many anatomic locations, may grow slowly or rapidly, and may result in death.

Cancers are often described by their growth characteristics and by the types of antigens displayed on their surface (**Part A**). Unfortunately, most tumors express differentiation antigens commonly found on normal cell types, although they are inappropriately or overly expressed on neoplastic cells. This property makes specific immunotherapy targeted to the tumor difficult without potentially harming normal cells bearing similar antigens.

Tumor-Associated Antigens

The efficacy of immune-mediated clearance of tumor cells is based on the expression of several types of tumor antigens on their surface. *Tumor-associated antigens (TAAs)* are unique in each individual bearing a specific neoplasm. Chemical carcinogen–induced cancers can express unique cell surface antigens between individuals and even between cell populations within an individual. Chemical carcinogens often cause random mutations in the cell, creating the novel expression of antigens. This phenomenon makes it difficult to design tumor vaccines or immunotherapies with antitumor antibodies for TAAs. The levels of TAAs can be used as a marker of therapy, as levels become elevated during progression of tumor growth.

In contrast, tumor antigens common for a specific neoplasm in all individuals are termed *tumor-specific antigens (TSAs)*. Tumors of suspected viral etiology often display similar tumor antigens. Although the role of viruses in tumorigenesis is not well understood or well documented, Epstein-Barr virus (EBV) has been associated with nasopharyngeal carcinoma and Burkitt's lymphoma. Other viral families such as herpesviruses and papillomavirus have also been linked to carcinomas.

There are two nonspecific TAA markers of tumor growth, termed *oncofetal antigens*, and are *α-fetoprotein (AFP)* and *carcinoembryonic antigen (CEA)*. Onco-fetal antigens are commonly expressed by fetal tissues during development in utero but may be aberrantly expressed by actively growing adult malignancies. Elevated serum AFP levels are most frequently associated with liver malignancies (hepatomas), prostate cancer, colon cancer, and teratomas, and in nonmalignant liver disease such as cirrhosis and hepatitis. Serum AFP levels often correlate with the efficacy of tumor therapy, with elevated levels occurring during active tumor growth.

CEA normally can be found in healthy human serum, and in the liver and pancreas during fetal development. In adults elevated CEA levels are closely associated with colon and pancreatic cancer (elevated in 70%–90% of cases) and in lesser frequency with breast, lung, and prostate cancer. Increased serum CEA levels may be found in other disorders of the lung and liver such as cirrhosis.

Immune Response to Tumor Antigens

Individuals are likely exposed to precancerous cells throughout life but they are effectively cleared by surveillance immune cells, primarily *natural killer (NK) cells* (**Part A**). For established solid tumors, *cytotoxic CD8+ T cells* are the principal mode of adaptive immune response. Antibody responses generally have little effect on clearing solid tumors. However, monoclonal antibodies specific for tumor markers and linked to a cytotoxic agent such as ricin can be effective at lysing tumor cells.

Antibodies can contribute to tumor cell destruction by mechanisms similar to the clearance of pathogens, namely *complement-mediated lysis* or *opsonization* of the tumor target with eventual destruction by Fc receptor–bearing cells (macrophages, neutrophils, or NK cells) (**Part B**). The binding of complement-fixing subclasses of Ig (IgM, IgG1, IgG2, and IgG3) initiates the *classical complement pathway*, causing tumor cell lysis by the terminal complement components C8 and C9.

Monoclonal antibodies to specific tumor antigens have also proved effective in some cases. *Anti-CD20*, a marker of B lymphocytes, is an FDA-approved therapy for B-cell lymphoma. Radioactively labeled forms of antibodies show improved tumor cytotoxicity, albeit with greater side effects. Monoclonal antibody therapy has also been effective for colon cancer and breast cancer (anti-HER2/neu antibodies).

NK cells also participate in the clearance of tumor cells by *antibody-dependent cellular cytotoxicity (ADCC)*. This mechanism is initiated by the opsonization of the tumor target with IgG1 or IgG3 subclass antibodies. By way of FcγRIII receptors, the NK cell binds and is triggered to release cytotoxic compounds in a manner similar to those released by cytotoxic T cells. Direct contact between the NK cell and the target cell is required for lysis. As indicated earlier, NK cells can also kill tumor cells, as measured in the laboratory, without prior exposure to the tumor or without ADCC.

Macrophages also have tumoricidal functions by binding to antibody-opsonized tumor cells via FcγRI and FcγRII receptors. Activated T cells in the surrounding milieu also secrete macrophage-activating compounds, *IFN-γ* and *TNF-α*. IFN-α is an activator of NK cells and directly suppresses some tumor cell growth by unknown mechanisms. IFN-α is approved therapy for hairy cell leukemia, myelogenous leukemia, Kaposi's sarcoma, and some lymphomas. TNF-α also enhances antitumor immunity by increasing the expression of adhesion of T cells to endothelium, allowing more effective infiltration of a tumor mass via its vasculature. Nitric oxide is released from macrophages that have been activated with IFN-γ, TNF-α, or lipopolysaccharide (LPS) from bacteria. Nitric oxide is a toxic oxygen compound with bactericidal and tumoricidal properties.

The administration of nonspecific macrophage-activating compounds also improves tumoricidal properties of macrophages. These compounds include IFN-α, *bacille Calmette-Guérin (BCG)*, an extract of bovine *Mycobacterium tuberculosis*, and muramyl dipeptide, a nonspecific macrophage activator. BCG is a component of the tuberculin skin test antigenic cocktail and also is used in immunization against tuberculosis in European communities. Its ability to nonspecifically activate macrophages has found use in the treatment of bladder cancer. Finally, IL-2, a product of activated Th1 T cells, also has antitumor activity and is approved therapy for some neoplasms.

T-cell cytotoxicity holds great promise in tumor therapy. Most tumors can provoke even a limited T-cell immune response supported by the isolation of antitumor T cells from the blood of patients or from within the tumor itself. These T cells, termed *tumor-infiltrating lymphocytes (TILs)*, can be stimulated in vitro with IL-2 and adoptively transferred back into the patient with significant tumoricidal effects. In vitro–activated lymphocytes are also called *lymphokine-activated killer cells (LAK cells)* and are now widely used in immunotherapies.

Tumor cells secrete several immunosuppressive agents that increase their survival and ability to evade immune recognition. Some tumor cells secrete TNF-β or IL-10, prostaglandin E_2, and AFP, all of which downregulate immune responses. In addition, tumors also evade immunity by constantly changing or mutating surface antigens.

16A Autoimmunity

A

Autoimmune Disease	Female:Male	HLA	Relative Risk
Ankylosing spondylitis	1:3	B27	88
Goodpasture's syndrome	1:1	DR2	16
Multiple sclerosis	10:1	DR2	10
Graves' disease	4:1	DR3	4
Myasthenia gravis	1:1	DR3	3
Systemic lupus erythematosus	8:1	DR3	6
Diabetes (IDDM)	1:1	DR3 or DR4	3
Rheumatoid arthritis	3:1	DR4	4
Hashimoto's thyroiditis	5:1	DR5	3

B

Autoimmune Disease	Autoantigen	Pathology
Autoantibody-mediated diseases		
Autoimmune hemolytic anemia	Rh blood group antigen on RBCs	RBC lysis, anemia
Systemic lupus erythematosus	DNA, snRNPs, histones, ribosomes, Ro/SSA, La/SSB	Glomerulitis, arthritis, vasculitis, skin lesions
Rheumatic fever	Cross-reactive cardiac myosin and streptococcal M cell wall antigen	Myocarditis, arthritis, fibrotic heart valves
Goodpasture's syndrome	Type IV collagen of basement membrane	Glomerulonephritis, pulmonary hemorrhage
Autoimmune thrombocytopenia purpura	Platelet integrin	Abnormal platelet functions, bleeding
Mixed essential cryoglobulinemia	IgG (rheumatiod factors)	Vasculitis
Pemphigus vulgaris	Cahedrin (epidermis)	Skin blistering
T-cell mediated diseases		
Multiple sclerosis, acute disseminated encephalomyelitis, experimental autoimmune encephalitis	Myelin basic protein, proteolipid protein	CNS paralysis, T-cell infiltration in brain
Diabetes (IDDM)	Pancreatic islet β cell	β-cell necrosis (lack of insulin production)
Rheumatoid arthritis	Unknown antigen in synovium	Joint inflammation, arthritis

The immune system has developed mechanisms for instructing cells of the immune system to ignore self-antigens and to respond only to foreign antigens. The failure of immune cells to ignore self-antigens may lead to autoimmune disease. Autoimmune diseases can be divided into those caused by T lymphocytes and those caused by antibody or immune complexes (**Part B**). Just as with the inflammation caused by pathogens, autoimmunity can elicit similar chronic inflammatory responses causing tissue injury. In theory, anyone may have susceptibility to autoimmune disease; however, strong MHC genetic associations have been linked to the development of some autoimmune syndromes (**Part A**). The association of MHC with a risk for autoimmunity is not surprising, since genes within the MHC directly influence T- and B-lymphocyte responses to both foreign antigen and self-antigen. These responses are based on the ability of proteins from particular MHC alleles to bind specific peptides, either foreign or self in origin. In the thymus, self-peptides bound to MHC class I and class II antigens orchestrate the deletion of autoreactive T cells based on the affinity of TCR-peptide-MHC interactions. Autoimmunity may result as a failure of central T-cell tolerance in the thymus.

HLA genes have been associated with autoimmune disease by comparing the frequency of particular genes in patients to their frequency in the normal population. Studies of monozygotic and dizygotic twins also demonstrate the importance of selected HLA genes in autoimmune susceptibility. For several autoimmune diseases, including multiple sclerosis (MS), rheumatoid arthritis (RA), type I diabetes, and systemic lupus erythematosus (SLE), the frequency of disease is approximately 20% to 35% in monozygotic twins, compared to less than 5% in dizygotic twins. Familial studies indicate that siblings sharing a particular HLA allele have significantly higher risks of developing the same autoimmune syndrome. Taken together, it is clear that the development of autoimmunity is a combination of both genetic and environmental factors. As indicated in **Part A**, HLA genes may play a major role in the risk of autoimmunity.

The HLA susceptibility for some diseases such as type I diabetes is partially understood at the molecular level. Individuals with the DR3/4 allele are protected from the development of diabetes when they have an aspartic acid residue at position 57 of the class II β chain. In contrast, individuals with diabetes often have serine, valine, or alanine at this same position. It is likely that these changes in the class II protein affect the binding and presentation of pancreatic autoantigens.

The initiating antigenic stimulus in most autoimmune diseases is unknown. It is unclear whether autoimmunity is started by self-antigens or by foreign antigens that have the appearance of self-peptides, called *molecular mimicry*. To date, no infectious agents have been clearly linked to the induction of autoimmune disease, with the exception of rheumatic fever. Based on amino acid sequences, it is clear that M proteins within the cell walls of streptococci have direct cross-reactivity with proteins in cardiac myosin. Antibodies elicited by streptococcal M proteins bind directly to cardiac myosin, causing pathology. The comparisons of antigens from pathogens with those from tissue proteins may reveal other links of molecular mimicry with autoimmune disease in the future. A second mechanism for initiating autoimmune disease may be the inappropriate release or metabolism of self-antigens from various tissues. For example, intracellular antigens such as those targets in SLE may be released from necrotic cells, resulting in their inappropriate presentation to the immune system. Moreover, virus infection of cells may either disturb the metabolism of tissues or cause modifications of self-proteins that are then able to elicit immune responses.

RA is characterized by chronic inflammation of the joints. It is linked to the HLA-DR4 gene and is more prevalent in women. RA is diagnosed by the presence of rheumatoid factor, antibodies directed against the Fc region of IgG. Both IgM and IgG class rheumatoid factors are found in patients' serum and synovial fluid. Immune complexes with complement activation cause tissue pathology in the joint and complement activation, which attracts macrophages, neutrophils, and T lymphocytes. Activation of macrophages and T cells in the joints causes the release of cytokines (TNF-α, IL-1, and IFN-γ), which amplify tissue damage. Rheumatoid factors may be elevated in other conditions such as chronic bacterial infection, viral infections, other chronic inflammatory diseases (SLE), and other autoimmune syndromes including mixed cryoglobulinemia, and hypergammaglobulinemia purpura. Joint deformities in RA are caused by the autoimmune-mediated destruction of cartilage and the erosion of bone.

SLE is a systemic autoimmune disease characterized by the production of autoantibodies to many intracellular autoantigens, primarily DNA and ribonucleoproteins. Autoantibodies to ribosomes, nucleoli, chromosomal proteins, and rheumatoid factors are also frequently observed in SLE.

Major clinical complications of SLE include immune complex–mediated kidney disease, central nervous system abnormalities (including seizures, neuropathies, and psychoses), and the characteristic butterfly rash and skin lesions. SLE afflicts primarily middle-aged women (8:1 male-female ratio).

Historically SLE was defined by the presence of the LE cell, a cell caused by opsonization of nuclear material by anti-DNA autoantibodies which is phagocytized by neutrophils. Virtually all SLE patients possess autoantibodies to double-stranded DNA or to one of the multiple other intracellular antigens, as determined by indirect immunofluorescence. Hypergammaglobulinemia can be observed with an increased consumption of serum complement, evidenced by lowered C3 and C4 levels.

For more information see Anderson, *IMS: Immunology*. Fence Creek, chapter 7.

16B Autoimmunity

A

Autoimmune Disease	Female:Male	HLA	Relative Risk
Ankylosing spondylitis	1:3	B27	88
Goodpasture's syndrome	1:1	DR2	16
Multiple sclerosis	10:1	DR2	10
Graves' disease	4:1	DR3	4
Myasthenia gravis	1:1	DR3	3
Systemic lupus erythematosus	8:1	DR3	6
Diabetes (IDDM)	1:1	DR3 or DR4	3
Rheumatoid arthritis	3:1	DR4	4
Hashimoto's thyroiditis	5:1	DR5	3

B

Autoimmune Disease	Autoantigen	Pathology
Autoantibody-mediated diseases		
Autoimmune hemolytic anemia	Rh blood group antigen on RBCs	RBC lysis, anemia
Systemic lupus erythematosus	DNA, snRNPs, histones, ribosomes, Ro/SSA, La/SSB	Glomerulitis, arthritis, vasculitis, skin lesions
Rheumatic fever	Cross-reactive cardiac myosin and streptococcal M cell wall antigen	Myocarditis, arthritis, fibrotic heart valves
Goodpasture's syndrome	Type IV collagen of basement membrane	Glomerulonephritis, pulmonary hemorrhage
Autoimmune thrombocytopenia purpura	Platelet integrin	Abnormal platelet functions, bleeding
Mixed essential cryoglobulinemia	IgG (rheumatiod factors)	Vasculitis
Pemphigus vulgaris	Cahedrin (epidermis)	Skin blistering
T-cell mediated diseases		
Multiple sclerosis, acute disseminated encephalomyelitis, experimental autoimmune encephalitis	Myelin basic protein, proteolipid protein	CNS paralysis, T-cell infiltration in brain
Diabetes (IDDM)	Pancreatic islet β cell	β-cell necrosis (lack of insulin production)
Rheumatoid arthritis	Unknown antigen in synovium	Joint inflammation, arthritis

MS is a neuromuscular autoimmune disease directed by T lymphocytes specific for various central nervous system autoantigens, including myelin basic protein. The clinical syndrome characterized by ataxia and motor weakness is due to T cell–mediated destruction of the myelin sheath, which surrounds nerve cell axons in the brain and spinal cord. Animal models suggest that Th1 T cells and perhaps CD8 T cells are required for the expression of MS. Although MS is a T cell–mediated disease, Ig concentrations may be elevated in the cerebrospinal fluid.

Acute disseminated encephalomyelitis is a related neuromuscular syndrome that may arise after influenza or measles infection, or some vaccinations with live attenuated virus. As in MS, T cells specific for myelin basic protein mediate this autoimmune response. While autoantibodies may arise as a result of T-cell help, they do not contribute to pathology. T cells and macrophages invade the central nervous system, contributing to inflammation of the myelin sheath. Early clinical symptoms include nausea, fever, back and neck stiffness, and headache. Individuals who survive the acute symptoms usually show no permanent neurologic damage.

Myasthenia gravis is an autoantibody-mediated disease characterized by muscle weakness and generalized fatigue. IgG class autoantibodies or immune complexes inhibit signaling by binding to the α chain of acetylcholine receptors. The receptors become internalized and degraded at the neuromuscular junction. As greater numbers of receptors are bound and internalized by antibody, progressive muscle weakness and dysfunction occur, and may be lethal.

Graves' disease is another syndrome characterized by autoantibody production to a cell surface receptor (as in myasthenia gravis). In general, the outcome of receptor binding by autoantibody may be either the inhibition or the stimulation of the target cell. While antibodies in myasthenia gravis inhibit receptor function, Graves' disease is marked by overactivation of receptors for thyroid-stimulating hormone (TSH). Under normal conditions, the pituitary gland secretes TSH, which binds to TSH receptor (TSHr) on the thyroid gland, causing the release of thyroid hormone. Feedback of thyroid hormone to the pituitary gland shuts off TSH production, thereby downregulating thyroid hormone release. Autoantibody binding to TSHr provides constant stimulation of the thyroid with constitutive release of thyroid hormone. The clinical symptoms of Graves' hyperthyroidism include weight loss, fatigue, nervousness, and sweating.

Insulin-dependent diabetes mellitus (IDDM), or type I diabetes, is caused by T cell–mediated destruction of insulin-producing islet of Langerhans' cells in the pancreas. Anti–islet cell autoantibodies are found in virtually all patients with IDDM, although the tissue pathology is T cell mediated. Individuals with HLA-DR3 or -DR4 have increased risk for this disease. As discussed earlier, those with an aspartic acid at position 57 of the β chain of the HLA class II molecule are protected from disease while those with a valine, serine, or alanine are at risk.

IDDM arises primarily in the teenage years and the onset is often preceded by viral infections (rubella, mumps, cytomegalovirus, or cocksackievirus). A viral etiology for this autoimmune disease has not been established unambiguously. Destruction of the β cells within the islet results in elevated blood glucose levels. Acute responses are marked by ketoacidosis while chronic symptoms in poorly stabilized individuals include renal and cardiovascular disease, neuropathies, and cataracts. IDDM patients require daily injections of insulin.

Hashimoto's thyroiditis is characterized by autoantibody production to thyroglobulin. It is a disease found primarily in women in the third to fifth decade of life and causes progressive hypothyroidism. Chronic disease leads to an enlarged thyroid goiter with lymphoid cell infiltration and fibrosis. The loss of immune tolerance to selected determinants of thyroglobulin is not well understood, although the clinical disease can be easily reproduced in animal models by immunization with thyroglobulin emulsified in adjuvants.

Autoimmune hemolytic anemia is marked by "warm" or "cold" antibodies to red blood cells (RBCs), causing anemia, fatigue, and splenomegaly. Anti-RBC antibodies specific to Rh determinants are detected by the Coombs' (antiglobulin) assay at 37°C. RBC lysis can be mediated by phagocytosis of Ig-bound RBCs or by complement-mediated lysis. A second group of anti-RBC antibodies, so-called *cold agglutinins*, are serologically reactive at 4°C and are typically complement-fixing IgM class autoantibodies.

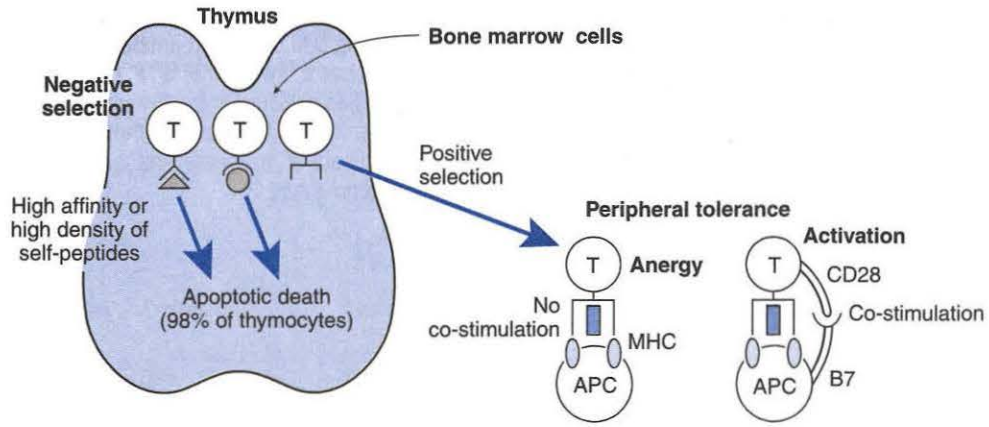
Goodpasture's syndrome is a rapidly fatal disease caused by autoantibodies directed at basement membrane structures in the kidney, lung, and small vessels. Symptoms are due to destroyed renal glomeruli (leading to hematuria and glomerulonephritis) and pulmonary hemorrhage.

17 Immune Tolerance

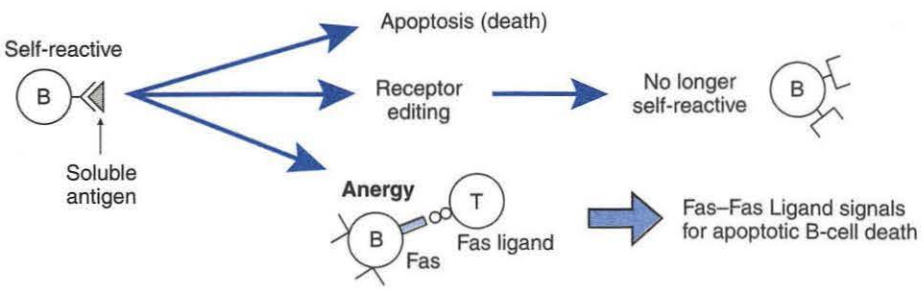
A Properties of Antigens That Affect Immunogenicity

Strong immunogen	Weak immunogen or tolerance
Intermediate dose	High or low dose
Particulate form	Soluble form
Large molecular weight	Low molecular weight
Strong MHC binding	Weak MHC binding
Subcutaneous route	Oral/intestinal route
Different from self-protein	Similar to self-protein

B T-cell tolerance



C B-cell tolerance



The immune system has several safeguard mechanisms in place to prevent effector cells of the immune system from attacking self-antigens and self-tissues. As described earlier, a major form of self-tolerance is achieved by the elimination of self-reactive B and T lymphocytes. For example, more than 98% of bone marrow cells that enter the thymus are eliminated and are presumed to be self-reactive. In a similar manner, B lymphocytes are deleted by inappropriate contact with self-antigens.

The induction of tolerance can occur at several levels within the immune system (**Figure**). Tolerance to self-proteins is important only for cells capable of antigen-specific immune responses, B and T lymphocytes. Other immune cells such as macrophages, granulocytes, and cells that present antigen (such as dendritic cells) have functional roles that do not discriminate between self-protein and foreign protein. Therefore, only B and T lymphocytes important in the adaptive immune response are susceptible to tolerance induction.

T-lymphocyte Tolerance

As described in detail in earlier chapters, T lymphocytes are activated by antigen presented on the surface of cells that express MHC class I or class II proteins. It is now understood that peptides may be expressed at a different density, depending on the type of specific MHC molecule. This phenomenon is important in tolerance since T lymphocytes are sensitive to both the **levels** of peptide it contacts and in the **affinity** of its T-cell receptor for the peptide-MHC complex (**Part B**). Peptides presented at low density on a cell are unable to activate T lymphocytes. In contrast, cells that express high levels of peptide with high affinity for T-cell receptor cause deletion or tolerance of the T lymphocyte. Intermediate levels of peptide are most efficient at stimulating T cells. Other specific biochemical features of proteins may affect their ability to elicit immune responses (**Part A**).

A second factor in T-lymphocyte tolerance is determined by the presence of *co-stimulatory molecules* on the antigen-presenting cell (APC) surface. Co-stimulatory molecules are accessory molecules that the T cell requires for full activation by peptide antigens. The best-defined co-stimulatory molecules are known as *B7-1* and *B7-2* and are found on dendritic cells, activated B cells, and macrophages. T cells possess specific surface receptors for B7 co-stimulation termed *CD28* and *CTLA-4*. T cells that recognize peptide in the absence of B7 co-stimulatory molecules become tolerant or anergic to the peptide. This mechanism may be important in tolerance to tissue antigens that are unable to be expressed in the thymus.

Some subsets of T cells also suppress ongoing immune responses. Suppressor T cells are characterized by expression of the CD8 surface molecule and are found to downregulate both B-cell responses and helper (CD4) T-cell responses. The mechanism of suppressor CD8 T-cell activity is not understood.

Immune tolerance can also be induced by administering antigen at abnormal levels. The continuous administration of low doses of an antigen is termed *low-dose tolerance*. Low-dose tolerance induction is the theory behind repeated injections of allergens into patients with specifically identified allergic responses. A second mechanism termed *oral tolerance* can be elicited by the oral administration of antigen. The mechanisms of oral tolerance are not entirely known; however, it is thought that cells in the intestinal mucosa process antigen in a tolerized form that downregulates subsequent exposure to the same systemic antigen. Oral tolerance to self-antigens such as insulin prevents diabetes in experimental animal models.

The presentation of antigen in **immunologically privileged** tissues (brain, eye, testes) does not elicit immune responses. The uterus is also a unique site in that the fetus fails to elicit immunity in the mother although it is technically a foreign tissue with foreign MHC molecules derived from the father. Immune privileged tissues do not have the usual perfusion by lymphatic vessels. In addition, specific cytokines released at these sites downregulate immune responses or activate regulatory T cells. Tissue barriers such as the blood-brain barrier often prevent T-cell migration to these sites unless infection alters these barriers.

B-cell Tolerance

B lymphocytes have several mechanisms for becoming tolerant to self-tissues. Once surface Ig is expressed on the B cell, the cell can be signaled to die by its interaction with antigen (called *clonal deletion*) or the B cell may change the specificity of its surface receptor to become non-autoreactive (called *receptor editing*) (**Part C**). Under normal conditions, B cells encountering antigenic stimulation can signal mutations in their Ig molecules that usually improve affinity for the antigen. On occasion, mutations in the Ig molecule may cause binding to self-antigens. When B cells bind self-antigens at high affinity, *apoptosis* (also known as *programmed cell death*) prevents the B cell from multiplying and becoming autoimmune. It is also known that B cells encountering self-antigen are unable to travel into T-cell areas of lymphoid tissues.

B cells can also be downregulated or *anergic* when they encounter antigen in a soluble form. An inability to cross-link surface Ig receptors by soluble monomeric antigen prevents B-cell activation. Finally, self-reactive B cells that encounter T cells bearing the Fas ligand protein are also signaled to die. The Fas and Fas ligand proteins are present on many cell types and have evolved specific signaling mechanisms that regulate apoptotic death and turnover under many natural situations.

Immune tolerance is mediated primarily by clonal elimination of B and T lymphocytes as originally proposed by Burnet. Burnet's original theory suggested that self-reactive lymphocytes must be eliminated during the development of immune cells. Clonal deletion of T cells is achieved by negative selection in the thymus, and that of B cells by several mechanisms in the bone marrow. In addition, the form of antigen presentation, both dose and affinity to MHC and surface receptors, controls immune tolerance. In general, antigens that interact at high affinity induce deletion or tolerance whereas antigens with intermediate affinity usually induce activation. Antigens in soluble form are usually least immunogenic whereas antigens that are insoluble and particulate in nature induce the best immune responses. Finally, tolerance can be disrupted by foreign infectious agents with components that are similar to those of self-proteins. Several infectious agents have been thought to elicit autoimmunity by this mechanism of molecular mimicry to self-proteins (see chapter on autoimmunity for greater detail).

Autoimmunity represents a failure of the mechanisms of tolerance that occur in the thymus and in peripheral tissues. Fortunately, abundant self-proteins efficiently induce both T- and B-cell tolerance. It is likely that autoimmunity may result when APCs that express co-stimulatory proteins also inappropriately acquire and present tissue-specific proteins. Lymphocytes then initiate an attack of the tissue similar to that seen on foreign antigens.

18 Immunodeficiency

Immunodeficiency Syndrome	Specific Defects	Immune Abnormalities
Bruton's X-linked agammaglobulinemia	Tyrosine kinase	Arrested B-cell development, no antibodies
Common variable hypogammaglobulinemia	Unknown	Decreased antibodies
Selective IgA deficiency	Unknown; MHC class III linked	IgA decreased
Transient hypogammaglobulinemia (infancy)	Unknown Th cell defects (?)	Transient decreases of IgG, IgA
X-linked hyper-IgM syndrome	CD40 ligand	Increased IgM, no isotype switching
DiGeorge syndrome	Thymus development	Decreased T cells
Bare lymphocyte syndrome	MHC class II	Decreased T cells and APC function, decreased humoral response
Ataxia telangiectasia	Kinase gene	T cells, antibody
Wiskcott-Aldrich syndrome	Glycosylated proteins	Decreased antibodies to glycosylated antigens
Severe combined immune deficiencies (4 types)		
ADA deficiency	Adenosine deaminase	Absence of B and T cells
PNP deficiency	Purine nucleotide phosphorylase	Absence of B and T cells
X-linked	Interleukin-2—receptors (IL-2R)	Absence of T cells
Autosomal recessive		Absence of B and T cells

Genetic defects that cause inherited immunodeficiency diseases exist at virtually all levels of the immune response, including those of the adaptive and innate immune systems. The diseases affect both B- and T-cell functions, complement or cytokine synthesis and release, phagocytic cell function, or cell surface receptor (**Table**). The first immunodeficiency disease, Bruton's X-linked agammaglobulinemia, was not identified until 1952. In the decades prior to this and before the development of antibiotic therapy, many infants died in early childhood owing to infectious diseases. Therefore, it was difficult to differentiate the lethal effects of infectious disease from those caused by immunodeficiency.

Most of the genetic defects of immunodeficiency are X chromosome–linked recessive traits. Therefore, males, having only a single X chromosome carrying the genetic defect, will be affected. In contrast, females who have the genetic defect on a single X chromosome will remain healthy because of the normal gene they possess on their other X chromosome. This fact accounts for the higher frequency of immunodeficiency diseases in males than in females (5:1). Immunodeficiency diseases can be divided into those affecting B-lymphocyte responses, those affecting T-lymphocyte responses, and those affecting both B- and T-cell responses. Other genetic defects interfere with phagocytic cell functions, cytokine or cytokine receptor signaling to cells, or the complement cascade.

B-cell Disorders

Bruton's X-linked agammaglobulinemia is marked by a block in B-cell maturation at the pre-B-cell developmental stage. Patients have extremely low serum levels of all Ig isotypes, an absence of germinal centers in the lymph nodes, few circulating B cells, and no plasma cell development. T-cell functions are essentially normal. The defective gene in Bruton's disease is located on the long arm of the X chromosome and encodes a defective protein, tyrosine kinase. The kinase activity is involved with the assembly of the pre-B-cell receptor to its surrogate light chains (Igα and Igβ). This deficiency disease is appar

ent in infants at the age of 6 months, the time at which maternal antibodies begin to disappear from the infants' circulation. Individuals with this disorder are given with intravenous human gammaglobulin for the treatment of bacterial infection.

Common variable hypogammaglobulinemia is also a condition of suppressed B-cell maturation resulting in low serum Ig levels. The syndrome does not appear until the second or third decade of life and the total number of circulating B cells is often normal. This congenital syndrome is not as severe as Bruton's disease; however, treatment is similar—the administration of pooled human gammaglobulin. The clinical presentation includes alopecia, malignancy, hemolytic anemia, and an increased incidence of bacterial infections and autoimmune diseases.

Selective IgA deficiency is the most common inherited Ig deficiency disease, found in approximately 1 in 800 individuals, primarily Caucasians of European descent. Patients with IgA deficiency may appear healthy, but often have an increased incidence of respiratory infections, as this Ig isotype is important to immunity in the lung. IgA deficiency has been linked to genetic defects in MHC class III genes and patients' higher incidence of atopy and autoimmune disorders, including systemic lupus erythematosus (SLE) and rheumatoid arthritis.

Transient hypogammaglobulinemia of infancy resembles Bruton's disease early in life. The syndrome is marked by a delay in IgG synthesis at the time when maternal Ig is leaving the infant's system. This transient Ig deficiency typically resolves by the age of 12 to 24 months. Infants may be prone to recurrent respiratory tract bacterial infections (gram-positive organisms) and infections of the skin and meninges.

T-cell Immunodeficiency

DiGeorge syndrome is characterized by a defect in the early development of thymic epithelium. This defect is expressed during embryogenesis as the third and fourth pharyngeal pouches are developing into the thymus and parathyroid glands. As a result, normal T-cell maturation and development in the thymus does not occur. While individuals may have normal Ig levels, antibody responses are defective because of the lack of helper T-cell functions. Patients with this syndrome express recurrent bacterial, viral, fungal, and protozoal infections. Delayed-type hypersensitivity is also impaired. Congenital defects contribute to an abnormal appearance (fish-shaped mouth, low-set ears) as well as cardiac abnormalities. The condition can be resolved by transplantation of fetal thymic tissue into the patient. Without transplantation, the condition generally resolves by the age of 6 years.

Bare lymphocyte syndrome is an immunodeficiency disease caused by a lack of MHC class II expression. The absence of MHC class II protein in the thymus prevents differentiation of CD4 T cells. Antigen-presenting cells are unable to present antigen to T lymphocytes in the absence of class II molecules on their cell surface. The genetic defect for this syndrome is found in regions that regulate class II gene transcription. The absence of CD4 T-cell activation also causes severe reductions in adaptive antibody responses. CD8 function and class I expression are normal.

Severe combined immunodeficiency disease (SCID). Some X-linked or autosomal recessive syndromes affect genes that cause defects in both B- and T-cell responses. Four different genetic disorders can lead to SCID. Defects in 2 different enzymes, each involved in purine catabolism, can cause the SCID response. The first defect is caused by the lack of adenosine deaminase (ADA) and the second one, by lack of purine nucleotide phosphorylase (PNP). The lack of either enzyme inhibits DNA synthesis and causes a buildup of intracellular products toxic to the lymphocyte. ADA deficiency increases intracellular deoxyadenosine triphosphate levels, inhibiting downstream DNA biosynthesis. The SCID condition is typically lethal by the second year of life, due to severe and recurrent microbial infections. Antibiotic and gammaglobulin treatment is prophylactic and bone marrow transplantation can reconstitute functional immune cells. The administration of purified ADA (bovine source) is also efficacious.

Wiskott-Aldrich syndrome is an X-linked disorder of a nuclear transcription factor. The defect is expressed in the thymus and spleen and interferes with normal platelet development and the ability to synthesize antibodies. Normal levels of IgG may be present with depressed levels of IgM antibodies, although B-cell numbers appear normal. T-cell immunity declines gradually over the course of the disease. The use of antibiotics and bone marrow therapy is an effective treatment for this syndrome.

Phagocytic Cell Deficiencies

A number of clinical syndromes or drug interactions can cause a decrease in the numbers of phagocytic cells. Autoantibodies against neutrophils or granulocytes, leukemia, the use of immunosuppressants or other drugs, and splenectomy can lead to depressed phagocytic cell populations.

Chronic granulomatous disease (CGD) is an X-linked recessive syndrome arising within the first 2 years of life. The disease is characterized by the systemic formation of granuloma at many sites and in many organs. The immunodeficiency is due to the inability of granulocytes to synthesize toxic oxygen radicals critical for the intracellular destruction of pathogens. The clinical syndrome is marked by recurrent infections with both gram-negative and gram-positive bacteria. Catalase production allows the organism to survive the small amount of toxic oxygen compounds made in the defective granulocyte. Treatment is prophylactic and includes antibiotic therapy, maintenance with adoptively transferred neutrophil populations, or both.

Other phagocytic diseases of lesser frequency include *glucose-6-phosphate dehydrogenase (G6PD) deficiency*, also an X-linked disease with an expression similar to chronic granulomatous disease. The condition interferes with the electron transport chain at the component NADPH. Another condition, known as *myeloperoxidase deficiency*, myeloperoxidase being an intracellular enzyme critical in killing microbes, interferes with normal neutrophil function. Patients have recurrent candida and staphylococcal infections caused by the inability of neutrophils to kill these organisms. *Chédiak-Higashi syndrome* is a rare disease interfering with neutrophil chemotaxis as well as the generation of lysosomal enzymes important for bacterial killing.

Finally, *complement deficiencies* have been observed for virtually all components of the complement cascade. The inability to fix complement allows microbes a survival advantage and patients have recurrent infections. A deficiency in the *inhibitor protein of C1 esterase (C1INH)* has been linked to hereditary angioedema. Unregulated C1s activity leads to increased capillary permeability and a transient localized tissue edema in the skin, GI tract, or respiratory tract. *C1q deficiency* also interferes with the initiation of the classical complement pathway and is found in SCID and hypogammaglobulinemic states. *C2 and C4 deficiencies* may provoke an SLE-like autoimmune disease due to the altered ability to clear immune complexes (via C4b) and resulting in glomerulonephritis. *C3 deficiency* is among the most severe of complement deficiencies because it contributes to opsonization, adherence, and inflammation (vascular permeability). Moreover, the chemotactic C5a fragment is not made in the absence of C3, impairing the influx of neutrophils and macrophages to the site of infection. Severe infections with *Neisseria* and *Streptococcus* species are common in C3 deficiency. The major defect in *C5 deficiency* is in the chemotaxis of phagocytic cells. Deficiencies in three terminal complement components, C6, C7, and C8, interfere with C′-mediated lysis of microorganisms. Recurrent infection with gonococcal and meningococcal species often causes sepsis and increased disseminated intravascular coagulation.

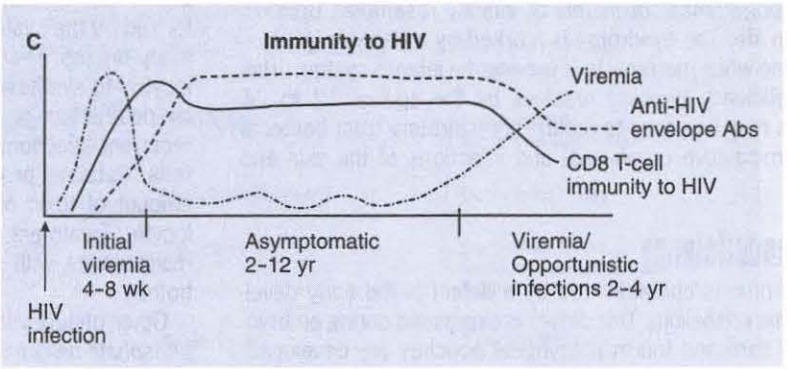
19 Acquired Immune Deficiency Syndrome (AIDS)

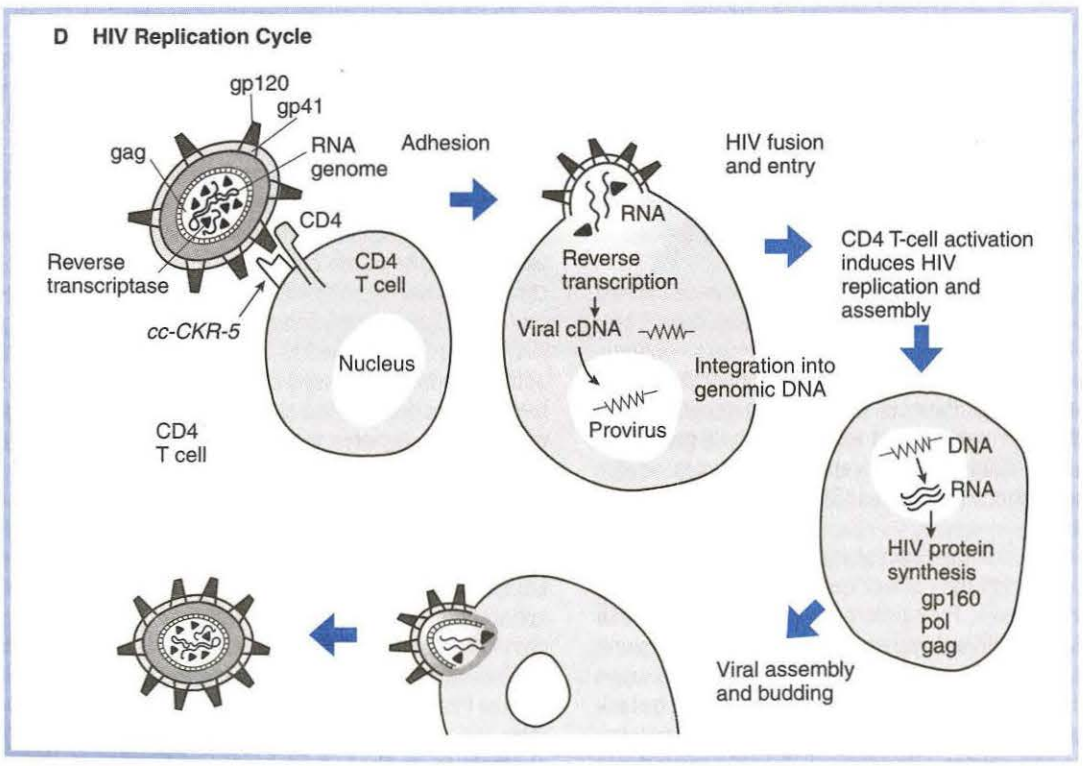
A Causes of Opportunistic Infections in HIV-infected Patients

Fungi	Bacteria	Viruses	Parasites
Pneumocystis carinii	*Mycobacterium tuberculosis*	Herpes simplex virus	*Toxoplasma*
Histoplasma capsulatum	*Mycobacterium avium-intracellulare*	Varicella-zoster virus	*Cryptosporidium*
Cryptococcus neoformans	*Salmonella* sp.	Cytomegalovirus	*Microsporidium*
Candida sp.			*Leishmania*
Coccidioides immitis			

B Malignancies of HIV Infection

Kaposi's sarcoma
Non-Hodgkin's lymphoma
Brain lymphoma
Burkitt's lymphoma





AIDS, first described in 1981, is caused by the transmission of the human immunodeficiency virus (HIV), a member of the Lentivirus family. Individuals particularly at risk include male homosexuals and intravenous drug users. The risk of infection from blood transfusions has been decreased, owing to the improved methods of detection of this virus and specific antibodies to HIV. At least 2 strains of HIV are known to elicit this disease: HIV-1 and HIV-2. The two strains are approximately 50% identical by nucleotide sequence, although the epidemiology for these viruses differs somewhat in that HIV-1 is most prevalent worldwide while HIV-2 is primarily found in West Africa.

Early AIDS is marked by fever and fatigue while chronic disease causes severe weight loss, lymphadenopathy, and recurrent opportunistic infections with *Candida*, *Pneumocystis*, *Mycobacteria*, and *Toxoplasma* species. Infants born to mothers infected with HIV are at high risk (25% infection rate for the newborn). Chronic viral infections at this stage include those with herpes simplex virus, Epstein-Barr virus, and cytomegalovirus. **Part A** lists agents that cause opportunistic infections in HIV-infected patients.

The tropism of HIV for CD4 helper T cells causes profound immune suppression (**Part C**). The continual decline in CD4 populations results in a reversal of the normal ratio of helper to suppressor T-cell subsets. Late disease will show a ratio of 1:2. The efficacy of treatment during the course of infection can be assessed by following CD4/CD8 T-cell ratios in the patient. After initial infection, a flulike illness is observed, followed by an asymptomatic phase that may last as long as 8 to 10 years before the onset of opportunistic infections. The HIV will infect both macrophages and CD4 T cells. Two HIV proteins, gp41 within the outer viral membrane and gp120 extending outside the viral coat, cause infection of the T cell by binding to the CD4 (**Part C**). The CC-CKR-5 receptor on CD4 T cells is normally bound by the chemokines RANTES, MIP-1α, and MIP-1β but also serves as a specific co-receptor for HIV binding and penetration. Recent studies suggest that some white individuals (approximately 1% of the population) possess mutations in the CC-CKR-5 chemokine receptor that may protect their T cells and macrophages from HIV infection. This observation may be responsible for the fact that some individuals harbor the HIV without ever showing the clinical complications of AIDS.

HIV is a member of the retrovirus family that transcribes its viral RNA into DNA inside the cell (**Part D**). The DNA copy is then integrated into the host cell genome as a provirus, where it causes latent and persistent infection before it replicates and infects other cells. A total of 9 genes comprise the entire HIV genome; 3 important genes are *gag*, *pol*, and *env*. The *env* gene is translated into the viral envelope proteins gp41 and gp120. The genes critical in replication and assembly have been targets in the design of drugs for HIV infection. Reverse transcriptase and viral protease are the targets of nucleoside analogues, zidovudine and dideoxyinosine, and protease inhibitors, respectively. While these drugs can reduce the transmission of virus into newly infected cells, the drugs do not clear the virus from cells with established infection. Clinically, the combination drug therapy causes a profound reduction in serum HIV titers as well as an increase in CD4 T-cell numbers. Since HIV must continually infect new cells, interfering in the viral cycle significantly improves disease prognosis. The existence of latent provirus necessitates continuous therapy throughout life. In addition, resistant viral strains have evolved, owing to the accumulation of mutations within the viral genome. Resistance to both protease inhibitors and inhibitors of reverse transcriptase can occur within days to months. The generation of mutations is a strategy that the virus uses to escape antibody and cell-mediated immunity.

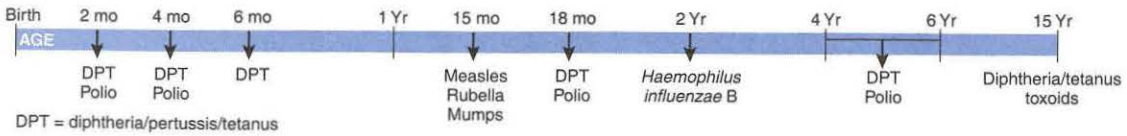
The adaptive immune response may slow the spreading of virus for a number of years (accounting for the latent and unsymptomatic early disease), although increases in HIV titers indicate that the immune response cannot effectively clear or contain the spread of this virus. Although viral titers are typically measured from the serum, the highest viral concentrations are found in lymph nodes, the site of high concentrations of CD4 T cells harbor the latent infection. The signals that trigger latent infected cells to replicate virus are not fully understood. Macrophages may be another important reservoir of infection where the virus is able to survive without intracellular killing. Interestingly, other functions of HIV-infected macrophages, including antigen-presenting cell function, may not be adversely affected.

20 Vaccinations

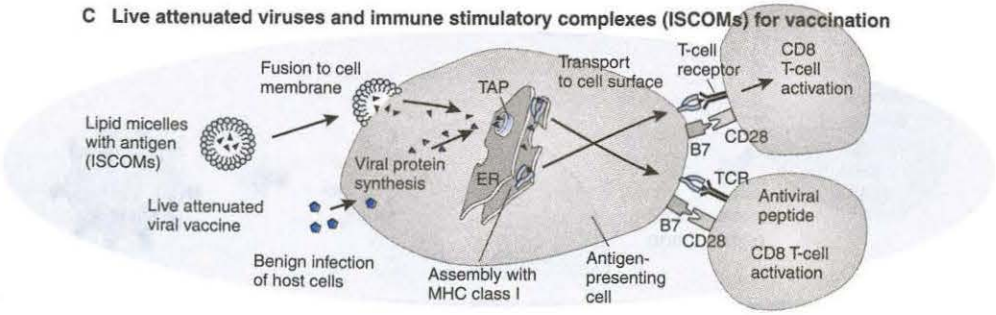
A Vaccinations

Vaccine	Pathogen/Clinical syndrome	Preparation/Usage
Diphtheria toxoid	*Corynebacterium diphtheriae*	Alum precipitated toxin; used in DPT
Tetanus toxoid	*Clostridium tetani*	Inactivated toxin; used in DPT; booster immunizations required
Pertussis	Whooping cough	Killed encapsulated bacterium; side effects of fever, convulsions, neurologic
Haemophilus influenzae B	Meningitis/epiglottitis in young	*H. influenzae* polysaccharide capsule
Typhoid	Typhoid fever	Heat-killed *Salmonella typhi*
Pneumococcal polysaccharide (Pneumovax)	*Streptococcus pneumoniae*	Polysaccharide capsule preparation of 14 strains of *S. pneumoniae* conjugated to carrier protein
Neisseria meningitidis	Meningococcal meningitis	Capsular carbohydrates of 4 strains (A,C,Y, and W-135)
Bacille Calmette-Guérin (BCG) (not used in U.S.)	Tuberculosis	Live attenuated *Mycobacterium bovis*; immunity invalidates DTH skin test in US
Borrelia burgdorferi	Lyme disease	Recombinant outer-surface protein of *B. burgdorferi*
Hepatitis B	Hepatitis	Recombinant hepatitis B surface antigen (HbsAg)
Polio	Poliomyelitis	1. Sabin oral vaccine; live attenuated virus; good intestinal and humoral immunity 2. Salk vaccine; formalin-inactivated virus; intramuscular humoral response but poor protection to intestinal infection
Rubella	German measles	Live attenuated virus grown in rabbit, duck, or human cell hosts
Measles		Live attenuated virus
Mumps		Live attenuated virus
Influenza	Influenza type A and B	Grown in chick embryo cells; formalin inactivated; viral antigenic shifts require new vaccines for protection
Rabies	Rabies virus	Two killed forms: 1. inactivated virus from duck embryo cells 2. virus grown in human diploid cells (preferred vaccine)
Typhus	Typhus	Formalin-killed *Rickettsia prowazekii* grown in chick embryos
Rocky Mountain spotted fever	—	Formalin-killed *Rickettsia rickettsii* grown in chick embryos

B



C Live attenuated viruses and immune stimulatory complexes (ISCOMs) for vaccination



The success of modern-day methods of vaccination is one of the most historically important contributions to human health, since infection by various pathogens is the leading cause of death worldwide. Edward Jenner performed early landmark studies in the late 1700s, utilizing a living cowpox virus vaccination to protect humans against the related smallpox virus. Louis Pasteur in the 1800s demonstrated that immunization with attenuated rabies virus would convey effective protection in humans against rabies virus infection. More recently, technologic advances have made available a vaccine for *Haemophilus influenzae* B (HIB), the leading cause of meningitis in children. The incidence of childhood meningitis from this organism has dropped 99% since introduction of the HIB vaccine in 1985. Although vaccines are available for many important infectious diseases (**Part A**) vaccines are *not* available for many of the world's leading causes of death by infectious agents including malaria, tuberculosis, schistosomiasis, respiratory diseases, and AIDS. **Part B** shows a typical immunization schedule for children.

Vaccination relies on the induction of effective adaptive immune responses, including CD8 T-cell, CD4 T-cell, and B-cell immunity. Preformed antibodies and specific T cells are critical in attacking infectious agents at their point of entry, such as mucosal surfaces in the lung. Methods for vaccination can be divided into 2 distinct types. The first type, *active immunization*, commonly employs vaccines composed of *antigenic extracts of pathogens*, or *killed* or *attenuated microorganisms* that are injected into the recipient. Antigenic extracts most effectively elicit antibody responses while live attenuated organisms can elicit both T-cell and B-cell immunity. Active immunization develops long-lived memory B- and T-cell immunity important for protection if reinfection occurs years after the immunization.

The second type of immunity that can be elicited to pathogens is *passive immunization*. Passive immunization utilizes antibodies preformed in an immune individual that are then transferred into the recipient. The recipient experiences immediate but short-lived immunity based on the transferred antibodies, which may consist of total Ig fractions from the donor or specifically purified antibodies. No memory B- or T-cell immune response is generated and immunity lasts only until Ig catabolism occurs. Serum Ig has an average half-life of approximately 3 weeks. Passive administration of total human gammaglobulin can provide good protection against common pathogenic infections in individuals with immune deficiency syndromes. Administration of antisera raised in other species (such as horses) can also be effective life-saving measures for tetanus or hepatitis B, although individuals run a risk of severe serum sickness. Serum sickness is a result of antibody responses directed against the foreign (horse) Ig molecules. Immune complexes form and precipitate in tissues such as the kidneys.

Purified antigen extracts are often not immunogenic unless mixed with *adjuvant*. For example, tetanus toxoid is only weakly immunogenic when administered without adjuvant. Adjuvants are compounds that enhance the immunogenicity of protein antigens, typically by making them insoluble in the host. Adjuvants may also work by nonspecific stimulation or manipulation of immune cells. A list of adjuvants includes alum, cytokines, oil emulsions, metalosalts, and lattices of antigenic complexes. Cytokines that have adjuvant properties include IL-1, IL-2, IL-12, and IFN-γ.

Immune responses can be generated with either killed organisms or living attenuated organisms. Living attenuated viral vaccines can provide the best B- and T-lymphocyte responses as well as longer-lived immunity. Live attenuated viral vaccines can enter host cells and synthesize peptides in the cytoplasm. Viral proteins are degraded in proteasomes and enter through TAP channels into the endoplasmic reticulum for eventual presentation by MHC class I molecules and activation of CD8 T-cell immunity. In contrast, killed organisms or antigenic extracts are unable to generate significant CD8 T-cell responses because they cannot synthesize intracytoplasmic proteins. Antigen extracts of pathogens are phagocytosed by antigen-presenting cells, such as macrophages and dendritic cells, for presentation with MHC class II molecules on the cell surface. CD4 (helper) T cells and antibody responses by B cells are elicited.

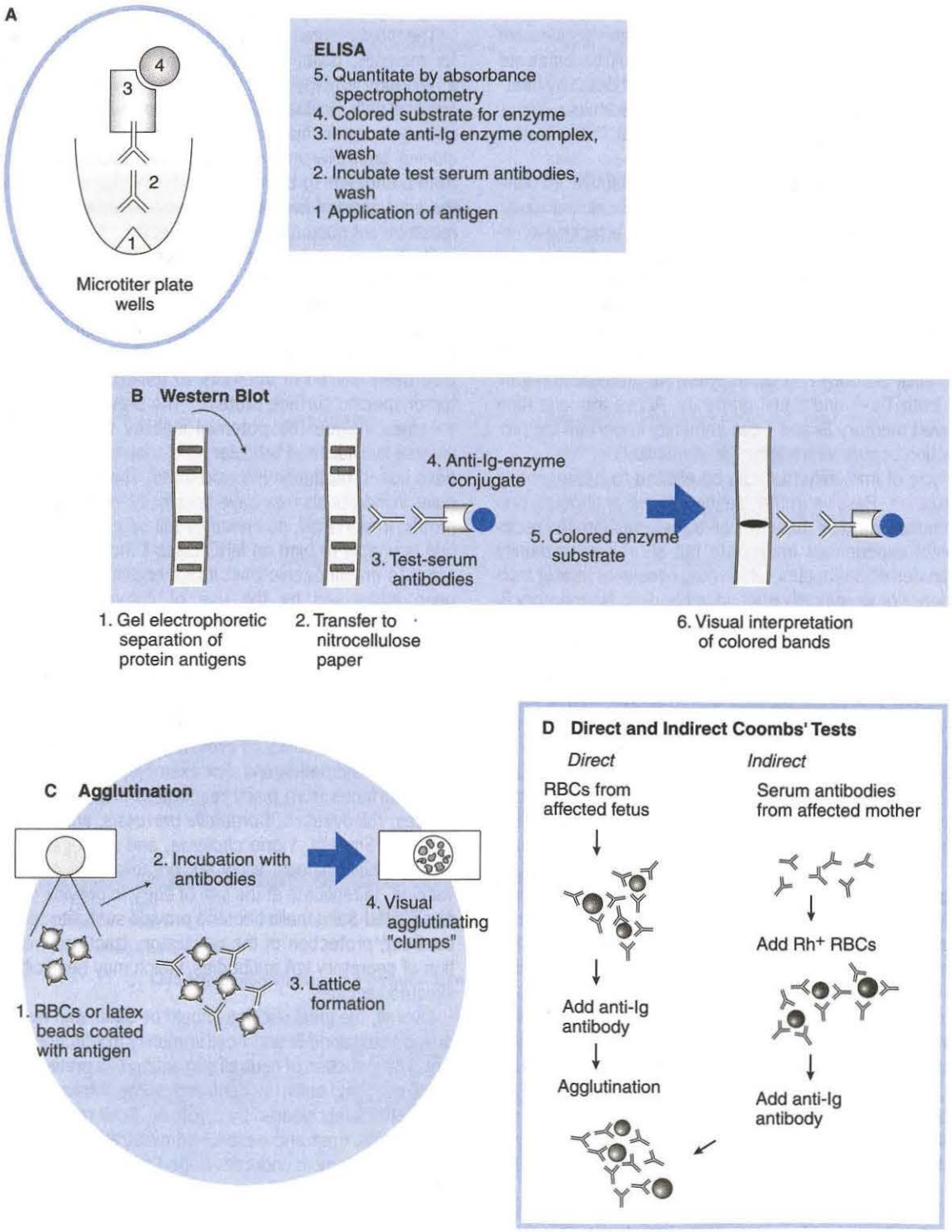
The most commonly utilized attenuated viral vaccines include those for measles, polio, rubella, mumps, and varicella. Methods of viral attenuation employ viral growth under various conditions in tissue-cultured cells. Mutations arising in the virus do not allow replication or pathogenicity in human cell substrates. Recent advances in DNA cloning technology have allowed the isolation of antigenic peptides from pathogens to be reproduced in benign viral vectors. In this way, the advantage of live vaccines can be preserved with the safety of a recombinant nonpathogenic viral vector.

Synthetic peptides of antigens are also used for vaccination. Of the many candidate antigenic sites, short stretches of peptides that confer protective immunity can be identified. For example, the pathogen of malaria, *Plasmodium falciparum*, has antigenic peptides that are effective in providing resistance to this infection. This approach has also been utilized in attempts to generate antitumor vaccines with tumor-specific surface proteins. The drawbacks of synthetic peptide vaccines include the potential inability to bind MHC molecules of diverse individuals. Particular MHC molecules are known to bind peptides based on the amino acid motif. Therefore, different MHC molecules in individuals may have specificity for different antigenic peptide motifs. Importantly, no immunity will be generated if a particular peptide is unable to bind an MHC class II molecule. In general, peptides are less immunogenic than intact organisms. These problems have been addressed by the use of immune stimulatory complexes (ISCOMs), which are synthetic liposome carriers of peptides that allow their greater entry into the cell cytoplasm (**Part C**).

Although pathogens themselves may have several routes of entry into the human host, vaccines are administered primarily by injection or as oral immunogens. The efficacy of vaccination has suffered because of an inability to provide adequate protection at the site of entry for most pathogens. For example, it is important to protect mucosal surfaces from many respiratory pathogens including influenza viruses, rhinoviruses, *Bordetella pertussis*, and bacteria such as *Salmonella*, *Shigella*, *Vibrio cholerae*, and pathogenic *Escherichia coli*. Ideal vaccines should allow the organism, as occurs with the polio vaccine, to replicate at the site of entry to provide surface protection. Attenuated *Salmonella* bacteria provide such site-specific immunity. In contrast, protection of the respiratory tract may require the generation of secretory IgA antibodies, which may be elicited only poorly by injected vaccines.

Overall, the ideal vaccine should be safe, with few side effects, yet provide sustained B- and T-cell immunity to infection with the live organism. The induction of neutralizing antibodies prevents many pathogens from infecting cells. In contrast, some intracellular pathogens are most effectively cleared by cytotoxic T-cell responses. Other factors, such as the cost and ease of administration, may be important factors, particularly in underdeveloped parts of the world.

21 Laboratory Methods in Immunology, Part I



The definition of immune function within individuals relies on the use of sensitive laboratory methods. This chapter defines the fundamental laboratory methods used for analysis of B- and T-lymphocyte responses, analysis of other hematopoietic cells (macrophages and neutrophils), and assessment of complement proteins. In addition, immune reagents can be used to identify the presence of antigens, such as blood group antigens on red blood cells (RBCs), or to identify the presence of pathogens.

Antibody Analysis

The humoral immune response is characterized by the secretion of antibodies by B lymphocytes after stimulation by pathogens or by an immunogen such as a vaccine. The presence of specific infectious agents can be measured by several techniques that can detect and identify specific antibody collected from the plasma of clotted blood. It is often important to measure the *specificity*, the *amount*, the *isotype* or subclass, and sometimes the *affinity* of antibody responses. All of these characteristics of antibody responses are regulated by the strength of the antigenic stimulation and the magnitude of B- and T-lymphocyte responses. Isotype analysis is important because distinct isotypes have different half-lives in the serum of individuals and different biologic functions (complement fixation, Fc receptor–binding properties, etc.). The affinity of specific antibodies may be important to define, as higher-affinity antibodies may clear antigens more effectively at lower concentrations. All of these parameters measure the overall efficacy of humoral responses to protect and clear the host of infection.

Enzyme-linked immunosorbent assay (ELISA) is one of the most commonly used and highly sensitive laboratory assays for the detection of specific antibodies or specific antigens. The ELISA is performed in the wells of plastic microtiter plates first incubated with specific antigens (such as pathogens) to which the presence of antibodies is to be determined (**Part A**). Patient serum is applied to the plate during an incubation period, followed by a washing step to remove unbound antibody. The presence of specific antibody will bind to the coated antigen and can be detected in a second incubation step using an enzyme-conjugated antibody specific for human Ig. The presence of this complex on the plate is identified using a color substrate able to react to the enzyme complex. The reaction is measured spectrophotometrically and can be designed both to quantitate the amount of antibody present and to identify specific Ig isotypes. The ELISA can also be modified to identify the presence of specific antigens or pathogens. In this case, specific antibody is first coated on the plate and used to trap specific antigens or pathogens from the patient sample. A second group of specific antibodies is then used to identify the presence of the trapped antigen. The assay is developed with a color-generating substrate as described earlier and can identify the presence of specific antigen in various tissue extracts or from serum. ELISA is used to detect the antibody response to many pathogens including HIV, hepatitis virus, rotavirus, streptococci, and *Borrelia burgdorferi*, and also to identify the presence of specific antibodies in autoimmune diseases such as anti-DNA and rheumatoid factor.

Radioimmunoassay (RIA) is designed in a similar manner as the ELISA with the exception that RIA uses radiolabeled antigen or antibody. The RIA can be performed in solution using a known amount of radiolabeled antigen mixed with unlabeled antigen together with the patient antibody sample. The antigens compete for binding to the antibody and the antigen-antibody complexes can be separated and quantitated by measurement of the radioactivity. The amount of radioactive binding to the patient sample is then compared to a standard curve based on known amounts of antibody and antigen. This comparison allows for quantitation of patient antibodies. The RIA has largely been replaced by nonradioactive assays such as ELISA.

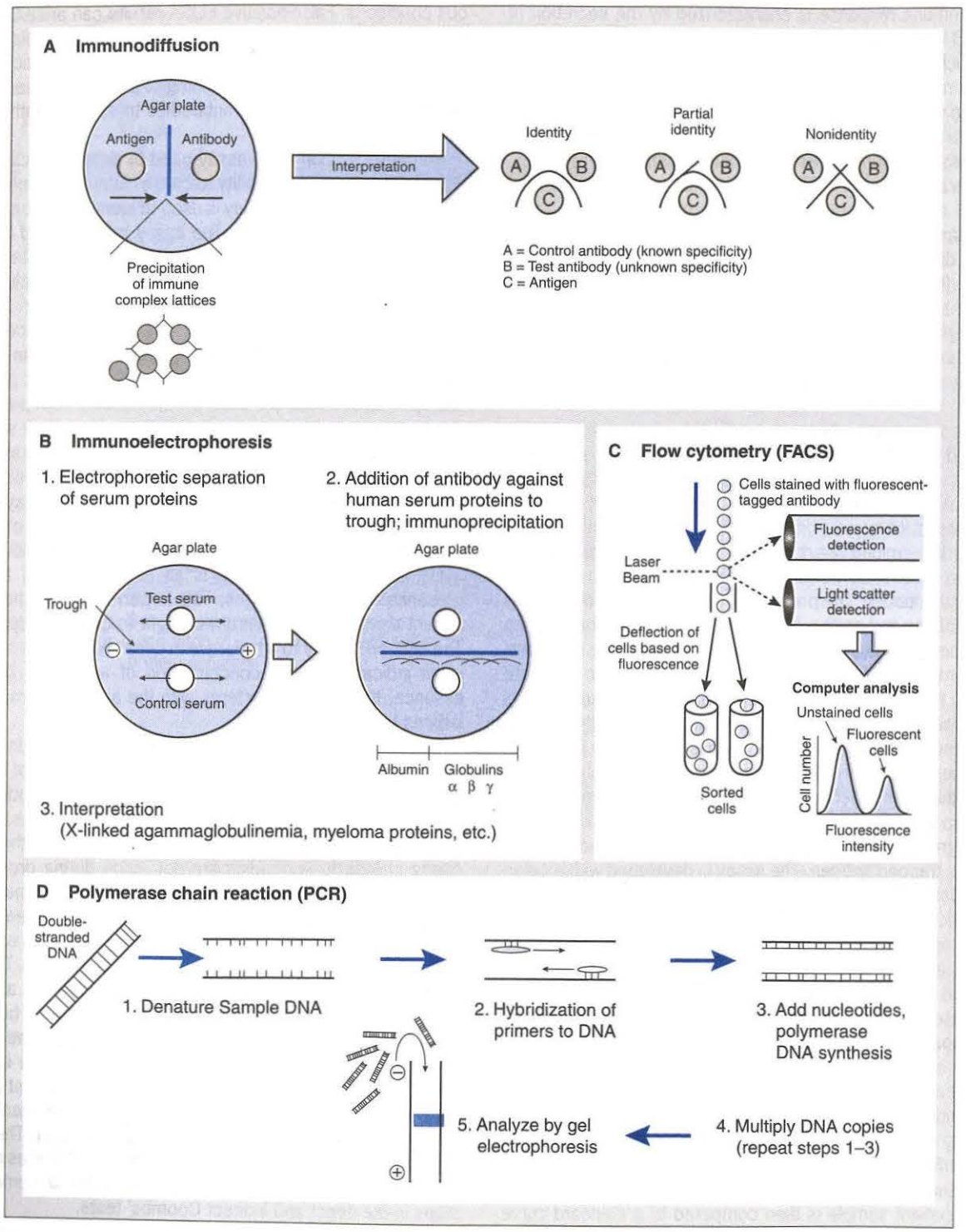
The *Western blot*, sometimes known as *immunoblot*, is an assay modified in design from the ELISA (**Part B**). In Western blots, antigens are first separated according to size by electrophoresis in polyacrylamide gels. This step allows for the detection of antibodies to a number of different antigens of specific molecular weights. After this step, the proteins are transferred to nitrocellulose paper and incubated with patient antibodies. Binding of antibodies to specific bands is detected, as in ELISA, with an enzyme-linked antihuman antibody followed by a color substrate. Blotting is advantageous compared to ELISA because the latter may demonstrate some false-positive reactions under various conditions. False-positive ELISA results can arise from sera from patients with hypergammaglobulinemia or rheumatoid factors, antibodies that can bind nonspecifically to plastic microtiter plates. Western blot can identify the binding to proteins of specific molecular weight. It is used to detect antibodies to specific pathogen proteins including HIV.

Hemagglutination is an assay used to detect specific antigens or antibodies based on the ability to cause clumping of specific antigenic particles (**Part C**). This assay is used to identify ABO blood group antigens in the typing of blood. The assay is performed by mixing test sera specific for the A or B blood group antigen with test RBCs. If the A antigen is expressed on RBCs, it will agglutinate or clump when anti-A antibodies are incubated. Agglutination is visible to the naked eye when performed on glass slides. The absence of specific antibody or antigen causes the particles or RBCs to stay in solution. The assay can be modified to detect antibodies to specific pathogens. For example, latex beads are coated with proteins from pathogens (such as streptococcus) followed by incubation with patient sera. The presence of antibodies indicates active infection as illustrated by agglutination of the latex particles. Its ease of use and lack of sophisticated equipment allow this test to be useful screening assays in physician offices. Agglutination, however, lacks the sensitivity of other assays such as ELISA, being as much as 100-fold less sensitive.

Hemagglutination inhibition is an assay designed to identify the presence of specific antigen. This is performed by the addition of a patient sample such as serum thought to contain a specific antigen. The antigen's ability to interfere with controlled agglutination reactions is an indication of the concentration of antigen in the sample. In essence, the antigen interferes with the ability to form agglutinating lattices between the beads.

The *Coombs' test (antiglobulin test)* is an assay originally developed to study erythroblastosis fetalis (hemolytic disease of the newborn). This disease occurs when mothers develop IgG antibodies directed at Rhesus (Rh) blood group antigens on RBCs of the fetus. Maternal antibodies are generated when fetal RBCs enter the mother's circulation during childbirth. Antibodies are not made during pregnancy with a first child but can develop during subsequent pregnancies with Rh incompatibility and when the mother has not been treated with RhoGam blocking antibodies. Anti-Rh antibodies do not agglutinate RBCs like antibodies to the ABO blood group antigens. Therefore, anti-Rh antibodies are detected using a second step of antihuman antibodies, which bind and agglutinate anti-Rh antibodies bound to RBCs. The direct Coombs' test utilizes fetal RBCs that potentially have been bound by maternal anti-Rh antibody. Fetal RBCs are incubated with anti-Ig, causing agglutination. The indirect Coombs' test utilizes maternal serum incubated with test RBCs followed by a second incubation with anti-Ig antibody, with subsequent agglutination. The indirect test determines the risk in the mother for passing antibodies causing erythroblastosis fetalis in future pregnancies. **Part D** demonstrates the steps in the direct and indirect Coombs' tests.

22 Laboratory Methods in Immunology, Part II



Immunofluorescence is an assay to determine the presence of specific antibodies or antigens in a test sample. It utilizes fluorescent-tagged antibodies visualized under ultraviolet microscopy. *Direct immunofluorescence* can be used to detect specific antigens such as the pathogen of syphilis, *Treponema pallidum*, or to detect *Legionella* species or *Mycoplasma pneumoniae*. In the direct assay, a tissue sample from the patient is incubated with antibodies specific to an individual organism that is linked with a fluorescent dye. Unbound antibodies are washed away and the sample is examined by microscopy. The presence of the organism is indicated by fluorescence due to the binding of specific control antibodies. *Indirect immunofluorescence (IIF)* is used to detect serum antibodies such as those to *T. pallidum* or antinuclear autoantibodies in diseases such as systemic lupus erythematosus (SLE). In this test, patient serum antibodies are incubated with slides coated with a specific antigen, such as *T. pallidum* or mammalian cells. The presence of specific antibodies to cellular antigens is detected by a second incubation with fluorescent-tagged anti–human Ig antibody. IIF is commonly used for the detection of anti-DNA and antinuclear antibodies found in SLE.

Antigens and antibodies can also be quantitated by the complement fixation test. The test utilizes the mechanism of complement-mediated lysis of test cells to detect levels of antigen or antibody. In a first step, antigen and antibody are incubated in the presence of complement proteins. The immune complexes fix a certain amount of complement in proportion to the number of immune complexes formed. The leftover, unfixed complement is measured by the addition of red blood cells (RBCs) coated with a hemolysin (anti-RBC) antibody. Residual complement in the assay lyses RBCs. The presence of original antibody or antigen in a test sample leaves little residual complement in the sample to lyse RBCs. Therefore, a low degree of RBC lysis indicates high levels of antibody or antigen in the test sample.

Other complement assays are also used to determine specific complement levels in patient serum. The hemolytic assay, also termed CH_{50}, is performed by adding patient serum complement to antibody-coated RBCs. A higher level of patient complement causes more RBC lysis in a quantitative manner. This test is used to detect complement deficiency diseases. ELISAs have also been developed to determine levels of individual complement proteins with the use of specific anti-complement antibodies.

Immunodiffusion is an assay to detect antigen-antibody complex that forms as a precipitation reaction visible to the eye. Immunodiffusion is performed in a semisolid agar medium using antigen and antibody in separated wells (**Part A**). Antibody and antigen diffuse through the agar toward each other and form a line of precipitation between the wells. The use of specific antibodies or antigen can help confirm specificity of the precipitation lines. The assay can be used with antigen extracts from pathogens combined with test serum or with nuclear cell extracts as antigen to detect the presence of autoantibodies such as those found in SLE.

Electrophoresis is a procedure that separates a complex mixture of proteins across an electric field based on their charge. This method can detect abnormal serum proteins associated with diseases such as myeloma, hypergammaglobulinemia, paraproteinemias, and Waldenström's macroglobulinemia. Immunoelectrophoresis combines this protein separation method with immunoprecipitation in agar (**Part B**). This method can define α, β, and specific γ globulins (IgA, IgM, and IgG) to help determine the levels of specific serum proteins.

Analysis of Cellular Immunity

B and T lymphocytes and phagocytic cells can be analyzed by several mechanisms. Assays can be performed to quantitate numbers of B-cell and T-cell subsets (CD4 versus CD8) as well as determine general cellular metabolism such as the synthesis and release of cytokines. These assays are particularly important in assessing immune deficiency disorders and determining specific allergies.

Delayed-type hypersensitivity (DTH) testing measures the T-cell response to individual antigens directly in the patient. The test is typically performed by the intradermal injection of specific antigens and is the basis for tuberculin skin testing. An area of induration and redness around the site of injection within 48 hours indicates a T-cell response to the particular antigen. The skin response results from both an infiltration of mononuclear cells and edema caused by the release of cytokines. This response is in contrast to erythema or the wheal-and-flare response, which occurs within hours of injection and is mediated by preformed antibodies.

The *nitroblue tetrazolium (NBT) test* is an assay for the phagocytic function of neutrophils. The test is important in the immune deficiency disease called chronic granulomatous disease (CGD). Normal-functioning neutrophils synthesize toxic oxygen compounds after activation, causing a yellow NBT dye to change dark blue and precipitate. All neutrophils from healthy individuals can be stimulated to produce the dark-blue formazan precipitate. Individuals with CGD do not metabolize oxygen radicals or hydrogen peroxide and are unable to kill intracellular organisms.

The phagocytic function of cells can also be tested in vitro by the addition of live bacterial cultures. The uptake and intracellular killing by phagocytic cells can be directly visualized and measured under a microscope. This test provides support for the NBT test in assessing the phagocytic function of neutrophils. Likewise, the ability of neutrophils to move toward sites of infection (chemotaxis) can also be measured in the laboratory using semisolid agar media.

Flow cytometry is a more recently developed technology used to quantitate numbers of specific cell subsets including B cells, T cells, and other immune cells. The technology, also called *fluorescence-activated cell sorting (FACS)*, is performed by staining live cell subsets from patients with specific monoclonal antibodies tagged with a fluorescent dye (**Part C**). The fluorescence-stained cells are passed through an instrument where cells are exposed to a laser beam that activates the fluorescent tag. Individual cells passing across the laser deflect light according to the fluorescence, which can be measured by detectors and interpreted by computers. The outcome of this technology is to accurately identify and quantitate individual cell populations. FACS can be particularly useful in syndromes such as AIDS where ratios of CD4 to CD8 T cells may be altered. Other lymphoproliferative disorders also can be assessed accurately by this technology.

Polymerase chain reaction (PCR) is the technology that identifies the presence of specific DNA molecules in a test sample. PCR is often used to detect the DNA of specific pathogens in tissues. It is performed by first purifying the DNA of sample tissue (**Part D**). Total DNA is incubated with short synthetic pieces of DNA complementary to the pathogen's DNA of interest. Precursors of DNA synthesis including nucleotides and polymerase are added to amplify the pathogenic DNA into thousands of copies. The amplified DNA is then electrophoresed in agar gels and identified according to its size and specificity. This technology is useful for its high sensitivity (ability to detect single strands of pathogenic DNA) and for its high degree of specificity. However, PCR requires highly sophisticated laboratory instrumentation and expertise.

QUESTIONS

1. Bone marrow is the source of precursor cells to:

(A) B and T lymphocytes

(B) macrophages

(C) fibroblasts

(D) neutrophils

(E) A, B, and D

2. Primary or central lymphoid organs differ from secondary or peripheral lymphoid tissues in the following way:

(A) Foreign antigen initiates the synthesis of lymphoid cells from central lymphoid organs

(B) Lymphocyte development requires antigen stimulation in peripheral lymph node tissues but not in central lymphoid tissues

(C) Lymphocyte development requires foreign antigen in both central and peripheral lymphoid tissues

(D) B and T lymphocytes require the same stimulus for development in central lymphoid organs

3. In peripheral lymphoid compartments:

(A) B lymphocytes develop into plasma cells and secrete antibody after activation with antigen in the germinal center

(B) Lymphocytes enter peripheral lymph nodes via the efferent lymphatic vessels

(C) The periarticular lymphoid sheets (PALS) are areas of B lymphocyte accumulation

(D) B and T lymphocytes remain in peripheral lymph nodes awaiting antigen stimulation and do not recirculate

4. B lymphocytes synthesize and secrete immunoglobulin and also have the following characteristics:

(A) Surface bound IgM and IgD serve as receptors for the uptake of antigen

(B) B lymphocytes have the capacity to process and present antigen on its surface with MHC Class II molecules to T lymphocytes

(C) Early B cell development occurs in the fetal liver

(D) All of the above

(E) Two of the above

5. Antigen specific immune responses are mediated by the following cell types:

(A) Eosinophils

(B) Monocytes

(C) B and T lymphocytes

(D) Fibroblasts

(E) Platelets

6. Bone marrow stem cells:

(A) Develop into lymphoid and epithelial cell lineages that later develop into all cells of the immune system

(B) Do not give rise to precursors of red blood cell development

(C) Require antigen stimulation before leaving the bone marrow to peripheral lymphoid organs

(D) Are precursor cells to myeloid stem cells that further develop into granulocytes, macrophages, platelets, and red blood cells

7. T lymphocyte development:

(A) Occurs primarily in the spleen

(B) Is characterized by the death of nearly 98% of bone marrow cells that arrive in the thymus

(C) Occurs in the absence of MHC Class I or Class II molecules

(D) Requires granulocytes for full maturation of cellular immunity

8. The specificity for antigen is found within what part of the Ig molecule?

(A) The combination of hinge region and papain sites on the heavy chains

(B) The hypervariable regions found on the Fab'2 fragments of the heavy and light chains

(C) The Fc domain that contains the CH2 and CH3 sites

(D) All of the above

9. Antigen or antigenic peptides are associated with B lymphocytes on the following molecules:

(A) Only surface IgM and surface IgD antigen receptors

(B) Only MHC Class I and Class II molecules

(C) Fc receptors on the surface of B lymphocytes

(D) Both surface IgM and IgD as well as surface Class I and II MHC molecules

(E) Complement receptor molecules

10. The following statements about immunoglobulin molecules are correct:

(A) IgM is found in highest concentration in the serum

(B) IgE is capable of activating the classic pathway of complement

(C) The J chain is found on all immunoglobulin isotype monomers

(D) Only isotypes of IgG are capable of crossing the placenta into the fetus

(E) The serum concentration of immunoglobulins does not change over the course of an active infection

11. Identify the statement about immunoglobulins that is not correct:

(A) IgE is the only isotype that binds and degranulates mast cells and basophils

(B) Secretory IgA is a principal defense mechanism against pathogens in the intestinal tract because it is secreted across epithelial cells and into mucus

(C) IgA is the earliest immunoglobulin produced in response to most infectious agents

(D) IgM is the only immunoglobulin isotype consisting of 5 molecules linked together by J chains

(E) Papain digestion of Ig molecules produces 2 Fab fragments and an intact Fc fragment

12. The structures of Ig responsible for binding specific epitopes on antigens are:

(A) Hypervariable regions of the heavy and light chains

(B) The constant regions of the heavy and light chains

(C) The hinge region and hypervariable region of the heavy chain

(D) The CH2 and CH3 segments of the heavy chain

(E) None of the above

For each statement listed below select the immunoglobulin that most closely describes its function (IgG, IgA, IgM, IgE, or IgD).

13. Associated with Type I hypersensitivity reactions (allergy).

14. Is able to cross the placenta into fetal tissues.

15. Is the predominant Ig isotype in mucosal secretions.

16. Is found as surface receptors on the lymphocytes.

17. Is the immunoglobulin that may be found as monomers or dimers in the serum.

18. Is found as the Ig of highest molecular weight due to its pentameric structure.

19. Identify the incorrect statement about the generation of immunoglobulin diversity:

(A) The light chain is composed of four distinct chain segments, the V, D, J, and C regions

(B) Regions of DNA are spliced out to bring V and J region segments of the light chain together

(C) Multiple copies of each V, D, and J genes are found in an individual's genome

(D) Isotype switching occurs when a contiguous V, D, J region combines with a different constant region gene

20. Identify the correct statement about B cell development:

(A) An IgG-producing B lymphocyte still has the ability to synthesize and secrete IgM

(B) Stimulation by antigen is first required to initiate gene rearrangement for Ig synthesis

(C) Ig gene rearrangements occur in the absence of any exposure to antigen

(D) Somatic mutation occurs before antigenic stimulation of the B lymphocyte

21. All but one of the following mechanisms can contribute to generating antibodies of many specificities:

(A) The splicing of one of many V, D, and J gene fragments of the heavy chain into one contiguous mRNA

(B) When a heavy chain of a defined specificity pairs with either the kappa or lambda light chain

(C) The addition of new nucleotides in the DNA between V and D or D and J gene segments contribute to antibody specificity

(D) Somatic mutation that arises within the variable region genes of the heavy and light chains

(E) The ability of plasma cells to contribute novel variable region genes for the synthesis of new specificities of immunoglobulin

22. All T lymphocytes in the peripheral circulation have the following cell surface molecule:

(A) MHC Class II

(B) Fc receptors

(C) CD3

(D) CD4

(E) CD8

23. Cytotoxic T lymphocytes (CD8 T cells) lyse target infected cells by the following mechanism:

(A) Secreting hydrogen peroxide into the target cell

(B) Complement mediated lysis

(C) Secreting perforin into the target cell membrane

(D) Binding the target with the T-cell receptor

24. Th1 and Th2 type CD4 T cells are characterized by the soluble lymphokines they secrete. The following cytokines are characteristic of Th1 and Th2 T cells:

(A) Th1 T cells secrete IFN-γ, IL-2, and TNF-α

(B) Th2 cells secrete IFN-γ and IL-2

(C) Th1 cells provide help for IgG synthesis by the secretion of IL-4

(D) Th1 and Th2 T cells both secrete IFN-γ and IL-2

25. Identify the incorrect statement about T-cell activation:

(A) T lymphocytes require two signals for activation, one by antigen with MHC Class I or II and a second by costimulation molecules

(B) The T-cell membrane protein, CD28, delivers a second activation signal to the T cell

(C) T-cell surface LFA-1 adhesion to ICAM on antigen presenting cells is critical for cell to cell contact and T cell activation

(D) T cells receive two signals, the first through the T-cell receptor and the second through B7 molecules on an APC that signals the T cell to become anergic (unresponsive)

26. CD8 T cells recognize peptide antigen in the following manner:

(A) Peptide antigens that are taken up by APCs from extracellular fluids and presented with MHC Class II molecules

(B) CD8 T cells recognize intracellular infected cells, such as viruses whose peptides are presented on the surface of APCs, with MHC Class I molecules

(C) Recognize viral peptides digested in the proteosome complex and presented with MHC Class II molecules on the surface of APCs

(D) Recognize either peptides engulfed from extracellular fluids or from intracellular infections presented by APCs with either MHC Class I or MHC Class II

27. Identify the incorrect statement about T cell receptor interactions with antigen presenting cells:

(A) The TCR resembles cell surface immunoglobulin Fab'2 fragments in structure and in function

(B) The CD4 molecule on T cells acts as a co-receptor because of its ability to interact with MHC Class II

(C) The CD8 molecule is a co-receptor with TCR for MHC Class I molecules

(D) Unlike immunoglobulin molecules, TCR generate only restricted specificities for antigen

28. Delayed type hypersensitivity (DTH) is regulated by the following cell type:

(A) Epithelial cells

(B) TH1 cells (CD4)

(C) TH2 cells (CD4)

(D) CD8 T cells

(E) Macrophages

29. Codominant expression of MHC molecules on the surface of cells is a reflection of two copies of an individual MHC gene, one on each chromosome. Codominant expression indicates that:

(A) MHC Class I and Class II proteins are expressed on the same cell

(B) A cell can express all three MHC Class I molecules: HLA-A, HLA-B, and HLA-C

(C) A cell can express two different gene products of the HLA-A protein

(D) A cell can express either of two proteins from the HLA-A gene complexes

30. Identify the incorrect statement about MHC molecules:

(A) Beta 2 microglobulin structures are found on both MHC Class I and Class II molecules

(B) MHC Class I molecules are found on virtually all nucleated cells, while MHC Class II molecules are primarily found on lymphocytes and other reticuloendothelial cells

(C) DR, DQ, and DP, are designations for human HLA Class II molecules

(D) Particular MHC Class I and Class II genes are associated with high risk of various autoimmune diseases such as multiple sclerosis and SLE

31. A patient is irradiated in order to receive bone marrow from a donor to reconstitute the immune system. The donor bone marrow cells are contaminated with mature B and T lymphocytes. The outcome of this bone marrow transplant will be:

(A) The transplant will be successful because the bone marrow will continue to grow in the host

(B) The transplant will not succeed because the remaining host cells will attack and reject the donor bone marrow

(C) The transplant will not be successful because mature donor bone marrow cells will reject the host tissue

(D) The donor cells will continue to grow along with remaining host lymphocytes resulting in mixed populations of cells

32. The complement immune response performs all of the following functions:

(A) Lysis of antibody coated target cells

(B) Lysis and anaphylaxis

(C) Lysis and activation of B lymphocytes

(D) Anaphylaxis, opsonization, chemotaxis of phagocytic cells, and lysis of target cells

33. Identify the incorrect statement about the complement pathway:

(A) The alternative pathway is activated by bacterial components, including LPS and yeast cell walls

(B) Complement components C3a, C4a, and C5a are anaphylotoxins and cause the contraction of smooth muscle fibers

(C) The Fc region of IgG and IgM molecules binds the first component of the complement system, C1

(D) The classic complement pathway is a component of the innate immune response

34. Identify the incorrect statement regarding cytokine functions:

(A) IL-2 is the principal growth factor for T lymphocytes

(B) TNF-α and IL-8 stimulate the expression of adhesion molecules, E-selectin, P-selectin, and ICAM

(C) TH-2 cytokines promote B-cell activation and the synthesis of IgG1 and IgE

(D) Disseminated intravascular coagulation causing a massive loss of plasma fluid into tissue spaces is due to overexpression of IFN-γ and IL-2

35. The cytokine that promotes neutrophil growth after an active inflammatory response is:

(A) GM-CSF

(B) IFN-γ

(C) IL-2

(D) TNF-α

(E) All of the above

36. The following immune cells are critical components of the innate or nonadaptive immune response:

(A) T lymphocytes

(B) B lymphocytes

(C) Fibroblasts

(D) Neutrophils

(E) All of the above

37. The following immune compounds or cells contribute to the innate immune response except for:

(A) C reactive protein (CRP) and mannan binding lectin (MBL)

(B) IgM antibody

(C) Natural killer cells

(D) B1 or CD5 B cells

(E) All of the above

38. Identify the inaccurate statement about phagocytosis:

(A) Pathogens taken up by neutrophils are killed by lysosomal granules including lysozyme and myeloperoxidase

(B) Fc receptors for Ig are important for the uptake of opsonized pathogens

(C) Interleukin receptors are important for the uptake of pathogens

(D) C3b bound to the surface of pathogens aids in the uptake by neutrophils by virtue of C3b receptors

39. All of the following immune responses are typical of Type I hypersensitivity except:

(A) IgM Class antibodies

(B) IgE Class antibodies bound to allergens

(C) The release of vasoactive amines by mast cells

(D) High affinity Fcε receptors present on mast cell membranes

(E) IL4 production by activated TH2 cells

40. The following clinical conditions are typical Type I hypersensitivity reactions except:

(A) Anaphylaxis

(B) Allergic rhinitis

(C) Cutaneous hypersensitivity

(D) Food allergy

(E) Serum sickness

41. Type II hypersensitivity reactions are characterized by:

(A) Antibody-complement mediated lysis

(B) TH2 lymphocytes

(C) The deposition of immune complexes

(D) Cytotoxic CD8 T cells

42. Type III hypersensitivity reactions are mediated by:

(A) Antibody-complement lyses

(B) TH2 T cells

(C) Deposition of immune complexes

(D) CD8 T lymphocytes

43. Type IV hypersensitivity, also known as delayed-type hypersensitivity, is an immune response found in contact hypersensitivity (poison ivy) and in tuberculin skin testing. This immune response is mediated by:

(A) Antibody-complement

(B) TH1 and/or cytotoxic T cells

(C) Deposition of immune complexes

(D) Neutrophils

44. Identify the one statement that is not correct about immunosuppression by corticosteroids:

(A) Corticosteroids directly inhibit antibody synthesis by B lymphocytes

(B) Cytokines that are downregulated by steroids are IL1, TNF-α, GM-CSF, IL3, IL4, IL5, and IL8

(C) Corticosteroids inhibit chemotaxis of cells and downregulate proinflammatory cytokine release

(D) Adhesion molecules such as E-selectin and P-selectin are downregulated by steroids

45. Identify the incorrect statement about immunosuppression:

(A) Nonsteroidal antiinflammatory drugs inhibit the cyclooxygenase pathway

(B) Cytotoxic drugs are immunosuppressive by inhibiting protein synthesis in the ribosome

(C) Cyclophosphamide and chlorambucil are alkylating agents that cross link DNA in the lymphocyte

(D) Cyclosporin A and FK 506 are microbial products that act by interfering with intracellular signaling molecules in the T lymphocyte

Match each of the following transplantation rejection immune responses to its appropriate statement:

46. Hyperacute rejection

47. Acute rejection

48. Chronic rejection

49. Graft-vs.-host disease

(A) Mediated by preformed antibodies to ABO antigens or MHC antigens

(B) Arises when donor T and B lymphocytes attack host MHC antigens

(C) Mediated by the host's antibody and cellular immunity directed against minor histocompatibility antigens on the transplant

(D) Occurs by T-cell activation to donor MHC or tissue antigens between 10 to 30 days after transplantation

(E) None of the above

50. Antibody-dependent cell-mediated cytotoxicity (ADCC) is characterized by:

(A) Opsonization by antibody or complement of a target tumor cell

(B) The uptake of target cells via Fc receptors on T lymphocytes

(C) Mediated by Fc and complement receptors on natural killer cells

(D) Terminal complement components C8 and C9 binding to the target cell

(E) Answers A and C above

Match each autoimmune syndrome with its most appropriate statement:

51. Systemic lupus erythematosus (SLE)

52. Multiple sclerosis

53. Myasthenia gravis

54. Insulin dependent diabetes mellitus (IDDM)

55. Goodpasture's syndrome

(A) Induced by IgG class autoantibodies directed at acetylcholine receptors

(B) Characterized by autoantibodies directed at double-stranded DNA and immune complex mediated kidney disease

(C) T lymphocyte mediated destruction of pancreatic Langerhans' cells

(D) Characterized by T lymphocyte destruction of myelin basic protein

(E) A rapidly fatal disease caused by autoantibodies directed at basement membrane structures in the kidney, lung and small vessels

56. Identify the incorrect statement about B and T lymphocyte tolerance:

(A) T-cell receptor stimulation in the absence of B7 costimulation leads to T-cell anergy

(B) B cells can modify autoreactive surface receptors to identify foreign antigens in a function known as receptor editing

(C) Immunologically privileged tissues, including the brain, the eye, and testis, are protected from B- and T-cell immunity due to several barriers, including inadequate lymphatic profusion or other physiologic barriers

(D) T cells undergo positive and negative selection in the spleen

Identify the following immunodeficiency disorders with its appropriate statement:

57. Bruton's α gammaglobulinemia

58. DiGeorge Syndrome

59. Severe combined immunodeficiency disease (SCID)

60. Wiskott-Aldrich Syndrome

61. Chronic granulomatous disease (CGD)

(A) Caused by a defect in the development of thymic epithelium leading to a deficit in normal T-cell maturation in the thymus.

(B) Caused by a defect in enzymes of purine catabolism leading to defects in both B- and T-cell responses. The defect is typically lethal within the first 2 years of life due to recurrent microbial infections.

(C) Caused by defective gene for protein tyrosine kinase on the X chromosome. The defect is marked by a block in B-cell maturation and patients have low serum levels of all immunoglobulin isotypes and few circulating B cells. T-cell functions are essentially normal.

(D) An X-linked recessive syndrome leading to the inability of granulocytes to synthesize toxic oxygen radicals important for the destruction of intracellular pathogens.

(E) An X-linked disorder expressed in the thymus and spleen and interferes with normal platelet development and the decreased ability of B lymphocytes to synthesize antibodies. B-cell numbers may be normal with the presence of normal levels of IgG but with depressed levels of IgM antibodies. T-cell immunity decreases during the course of disease.

62. In analysis of antibody responses, Western immunoblot has the following advantage over other laboratory tests:

(A) Increased sensitivity of immunoblotting as compared to ELISA

(B) Can identify antibody responses to proteins of specific molecular weight within pathogens

(C) Can identify specific T lymphocyte immune responses

(D) Utilizes a fluorescent tagged second antibody specific for human Ig

63. Polymerase Chain Reaction (PCR) is useful for the following reason:

(A) PCR can identify specific immunoglobulin isotypes to pathogenic organisms

(B) PCR utilizes the electrophoresis of proteins from pathogens

(C) PCR can identify the specific DNA of a pathogen by its amplification using nucleotide primers specific for DNA

(D) PCR is used in identifying immune deficiency disorders where antibody or responses are inhibited

ANSWERS AND EXPLANATIONS

1. **The answer is E.**

All cells specific to the lymphoid system derive from bone marrow cells. Other cells such as fibroblasts or epithelial cells do not originate from bone marrow.

2. **The answer is B.**

Lymphocyte development typically occurs in the absence of foreign antigen in the thymus and bone marrow unless active infection is present. In contrast, peripheral lymph nodes are a reservoir for local antigens caused by infection that stimulate antigen specific B- and T-lymphocyte responses.

3. **The answer is A.**

Germinal centers are the site of B-cell development into antibody secreting plasma cells. B and T lymphocytes continuously recirculate throughout the body and into various peripheral lymphoid organs.

4. **The answer is D.**

Antibody secreting B lymphocytes, termed plasma cells, are the end stage development of B lymphocytes. However, B lymphocytes also present short peptide antigens to stimulate T-cell responses. Finally, neonatal B-cell development occurs in the liver, a function that is maintained in the bone marrow shortly after birth.

5. **The answer is C.**

The adaptive or antigen specific immune responses are mediated by

B lymphocytes and by specific receptors on the surfaces of T lymphocytes.

6. **The answer is D.**

Granulocytes (neutrophils, eosinophils, basophils) as well as macrophages, platelets, and RBCs all originate from the myeloid stem cell.

7. **The answer is B.**

Bone marrow cells that enter the thymus undergo positive and negative selection based on the interaction of cells with MHC Class I and Class II proteins. The result of selection in the thymus causes the development of both CD4 and CD8 single positive T lymphocytes.

8. **The answer is B.**

Antigen specificity is determined by unique amino acid sequences within the hypervariable regions of both the light chain and the heavy chain Fab'2 fragments. The hinge region provides flexibility to the antibody molecule while the Fc region of Ig has other important biological functions including the binding to Fc receptors on macrophages and neutrophils.

9. **The answer is D.**

Both surface IgM and surface IgD have antigen-binding properties that stimulate the B lymphocyte, followed by internalization and processing of the antigen. Processed antigen returns to the cell surface in the form of peptide complex to MHC Class II molecules. Finally, B cells infected with virus can process and transport peptides to the surface with MHC Class I molecules.

10. **The answer is D.**

Only isotypes of IgG are capable of crossing the placental barrier because of size and other limitations.

11. **The answer is C.**

The earliest response of antibodies is typically of the IgM isotype followed in time by isotype switching to the IgG subclasses.

12. **The answer is A.**

The hypervariable sites of both the heavy and light chains confer specificity of antibody molecules for the epitopes on antigenic proteins. Constant region amino acid sequences primarily confer structural features to the Ig molecule.

13. **The answer is D.**

IgE is responsible for the binding and degranulation of mast cells and basophils with the release of vasoactive amines.

14. **The answer is A.**

All IgG isotypes readily cross the placenta into the fetal circulation and provide transient protection from infection in the newborn child.

15. **The answer is G.**

Secretory IgA is the predominant isotype found in mucosal tissues protecting from respiratory and intestinal tract infections.

16. **The answer is F.**

B lymphocytes have membrane bound IgM and IgD as antigen-binding receptors on their surface. These receptors activate the B cell after exposure to antigen.

17. **The answer is B.**

IgA may be found as a monomer in the serum or as a dimer (two molecules) connected by a J chain.

18. **The answer is C.**

IgM has the highest molecular weight because it is five Ig molecules interconnected by J chains.

19. **The answer is A.**

The light chain polypeptide is composed of genes from the variable (V), joining (J), and constant region (C). The D, or diversity, segment is found only as a component of the heavy chain polypeptide.

20. **The answer is C.**

Antibody genes rearrange in the B cell in the absence of antigenic stimulation. The generation of the Ig repertoire occurs by random association of V, D, and J region genes. As these genes are spliced together copies of the gene are lost by the B cell. For example, an IgG secreting B cell has already spliced out and lost its ability to synthesize and secrete IgM.

21. **The answer is E.**

Plasma cells are end-stage differentiated cells that no longer rearrange immunoglobulin genes.

22. **The answer is C.**

CD3 is a collection of accessory molecules important for T-cell receptor signal transduction and is a marker of all T lymphocytes. Only helper T cells possess CD4 molecules, while cytotoxic T cells possess the unique CD8 molecule.

23. **The answer is C.**

CD8 T cells secrete pore-forming molecules, perforins, which punch holes in the target cell membrane, causing lysis.

24. **The answer is A.**

Th1 T cells provide help for IgG_2 isotype synthesis by B lymphocytes and also provide help to other T cells (CD8 T cells). Th1 cells are characterized by secretion of IFN-γ, IL-2, and TNF-α. Th2 T cells secrete IL-4, IL-5, IL-6, and IL-10.

25. **The answer is D.**

T cells receiving only signal one through the T-cell receptor become anergic to the specific antigen. All T cells, CD4 and CD8, require signaling through the TCR and CD28 molecules for activation.

26. **The answer is B.**

CD8 T lymphocytes primarily recognize peptides of intracellular infections that are digested in the proteosome and presented with MHC Class I molecules on the APC surface.

27. **The answer is D.**

The assembly of one of multiple TCR region genes generate highly diverse specificities much like the assembly of immunoglobulin genes. The CD4 and CD8 molecules act to enhance TCR-MHC interactions in cell-to-cell contacts.

28. **The answer is B.**

CD4 TH1 type T cells that secrete IFN-γ are responsible for DTH responses. The classic DTH response is in skin testing for tuberculin that reveals a characteristic erythema response within 24 hours after the injection of antigen under the skin.

29. **The answer is C.**

Codominance indicates that the HLA-A gene expressed on each of the two chromosomes are both translated and expressed at the surface of somatic cells. Allelic exclusion, the silencing of one copy of a particular allele, does not occur for MHC genes.

30. **The answer is A.**

Beta 2 microglobulin is a polypeptide chain only associated with MHC Class I molecules. MHC Class II have two polypeptide chains termed alpha and beta.

31. **The answer is C.**

Mature lymphocytes in the donor bone marrow will recognize and attack host cells because of MHC differences in a classic example of graft-vs-host disease. This response can be avoided by a careful depletion of donor bone marrow of mature lymphocytes.

32. **The answer is D.**

The complement system has several biologic functions, including the lysis of antibody coated target cells, the degranulation of mast cells to release histamines, the opsonization of target cells for uptake by phagocytic cells, and the attraction of other inflammatory cells to a site of pathology.

33. **The answer is D.**

The classic complement system is a component of the adaptive immune response because the first complement component, C1, binds IgM or IgG molecules that have been synthesized in response to antigen stimulation of B lymphocytes.

34. **The answer is D.**

DIC is caused by systemic effects of TNF-α causing increased vascular permeability and a loss of plasma fluids. DIC is a consequence of pathogens in the blood stream (septic shock).

35. **The answer is A.**

GM-CSF (granulocyte macrophage–colony stimulating factor) is a growth factor for neutrophils.

36. **The answer is D.**

Neutrophils constitute one component of the nonspecific immune responses that aids in the clearance of pathogens.

37. **The answer is B.**

IgM is a response of specific antigen stimulation by B cells and, therefore, constitutes the adaptive immune response. In contrast, CD5 B cells, also known as B1 B cells, secrete cross-reactive antibody that binds many bacterial proteins. CRP and MBL are nonspecific antibacterial proteins that act in opsonizing target pathogens.

38. **The answer is C.**

Interleukin receptors are not required for the phagocytosis of foreign antigens. In contrast, C3b and IgG opsonized pathogens are readily taken up by Fc receptors and C3b receptors, respectively.

39. **The answer is A.**

IgM is not a component of Type I (allergic) responses. IL4 production by TH2 cells promotes IgE synthesis by B lymphocytes. Mast cells degranulate after IgE is cross-linked by antigen bound to Fcε receptors.

40. **The answer is E.**

Serum sickness is classic Type III hypersensitivity caused by the formation of immune complexes.

41. **The answer is A.**

Antibody and complement mediated lysis of target cells is a common mechanism of Type II hypersensitivity. Transfusion reactions, hemolytic anemia, and erythroblastosis fetalis are all Type II hypersensitivity conditions.

42. **The answer is C.**

The deposition of immune complexes initiate the typical Type III immune response known as Arthus reactions. Complement activation by immune complexes further initiates inflammation and the attraction of neutrophils and fluids to the site due to increased vascular permeability.

43. **The answer is B.**

TH1 and/or CTLs are responsible for Type IV hypersensitivity. The activation of TH1 T cells by antigen causes the release of chemokines and lymphokines that attract macrophages, initiate tissue destruction, and increase adhesion molecules at the site of pathology.

44. **The answer is A.**

Corticosteroids do not have direct effects on B lymphocyte immunoglobulin synthesis.

45. **The answer is B.**

Cytotoxic drugs are immunosuppressive by the inhibition of DNA synthesis in lymphocytes. Examples of DNA synthesis inhibitors are cyclophosphamide, chlorambucil, and methotrexate.

46. **The answer is A.**

47. **The answer is D.**

48. **The answer is C.**

49. **The answer is B.**

50. **The answer is E.**

ADCC occurs by the attack of target cells (such as tumor cells) coated by IgG1 or IgG3 Class antibodies. Uptake by the NK cell is mediated by FcγR III receptors. ADCC can also be mediated by complement opsonized targets with uptake through complement receptors (C3b receptors).

51. **The answer is B.**

52. **The answer is D.**

53. **The answer is A.**

54. **The answer is C.**

55. **The answer is E.**

56. **The answer is D.**

Positive and negative selection occur in the thymus where greater than 90% of incoming bone marrow cells are deleted due to autoreactivity to self-tissues.

57. **The answer is C.**

58. **The answer is A.**

59. **The answer is B.**

60. **The answer is E.**

61. **The answer is D.**

62. **The answer is B.**

Western immunoblot has the basic advantage of identifying antibody responses to specific pathogen proteins. It helps rule out potential nonspecific binding that can occur in other diagnostic assays such as ELISA.

63. **The answer is C.**

Polymerase chain reaction (PCR) is performed by amplifying the specific DNA of a pathogen out of a clinical specimen. The test is performed using specific primers to the DNA of the suspected pathogen followed by many rounds of amplification utilizing DNA polymerase and nucleotides. The amplified DNA is electrophoresed in agar gels and identified by its size and specificity.

Glossary

ABO antigens are expressed on red blood cells and used for the typing of human blood. Individuals express antibodies to the blood group, either A or B, that are not expressed on their own red blood cells.

Acquired immune deficiency syndrome (AIDS) is an immunodeficiency disease caused by the infection of CD4 T cells and macrophages with the human immunodeficiency virus (HIV).

Acute lymphoblastic leukemia is a malignancy of undifferentiated lymphoid cells that replicate in an uncontrolled manner.

Adaptive immune response (or acquired immune response) describes the immunity to antigens that requires the selection of antigen-specific lymphocytes (both B and T lymphocytes) and the generation of memory cells. This response is in contrast to innate or non-adaptive immunity, which is not regulated by antigen-specific cells.

Adenosine deaminase deficiency is a common cause of severe combined immune deficiency disorders and results in a buildup of purine nucleotides (adenine and guanine) with toxicity to the cell.

Adhesion molecules are a collection of cell surface proteins including integrins, selectins, and related proteins that mediate the rolling of lymphocytes along vascular tissues and homing to sites of infection (chemoattraction).

Adjuvant is a compound that is mixed to an antigen, enhancing its immunity. Adjuvant is commonly used in vaccines and in immunization of experimental animals for immune responses.

Affinity is the attraction or binding strength of one molecule such as an antibody or a T-cell receptor for another molecule such as an antigen.

Affinity maturation describes the increase in antibody affinity for antigen over the time frame of an immune response. Affinity matures or increases dramatically in secondary exposure to antigen.

Allergens are foreign particulate antigens that induce allergic reactions (atopy) or hypersensitivity.

Allergic reaction is an immune response mediated by T cells or IgE antibody that typically cause the release of vasoactive amines from mast cells. Common types of allergic reactions include hay fever and asthma.

Allogeneic describes the differences in individual alleles for MHC molecules. The rejection of transplanted tissues is often based on allogeneic MHC differences between the transplanted tissue and the recipient host.

Allotypes are unique polymorphisms found on the Fc region of the immunoglobulin heavy chain. Allotypes are characteristic of individuals or animal strains and can be identified with the use of specific antibodies directed at the allotypic epitope.

Alternative pathway describes the activation of the complement cascade when C3b is bound directly to the surface of a pathogen. The alternative pathway is not activated by antibody-antigen complexes.

Anaphylactic shock results from severe systemic allergic reactions mediated by IgE–mast cell interactions, resulting in the systemic release of vasoactive molecules. The response may trigger systemic vascular collapse with major organ damage or suffocation due to tracheal inflammation.

Anaphylatoxins are the C5a, C3a, and C4a complement cleavage products that mediate a release of vasoactive compounds, histamine and serotonin, from mast cells and basophils. Anaphylatoxins result in smooth muscle contraction, attract inflammatory cells, and cause an influx of fluids to a site of tissue infection.

Anergy is the inability of B or T cells to respond to an antigen.

Antibodies are protein products of B lymphocytes that specifically bind antigen and mediate a variety of responses including clearance of the pathogen or endocytosis by other cells. Individual antibody isotypes have unique biologic functions including complement fixation, binding to Fc receptors, and acting as cell surface antigen receptors.

Antibody-dependent cell-mediated cytoxicity (ADCC) occurs when phagocytic cells with Fc receptors kill antibody-coated cells or targets. Natural killer (NK) cells are important cells that function in ADCC.

Antigenic determinant, also known as *epitope*, is the portion of an antigen that binds specifically to antibody-combining sites or T-cell receptor sites.

Antigenic drift describes the mutations that occur in bacterial or viral proteins (such as influenza), allowing the pathogen to escape immune detection.

Antigenic shift is an exchange of genetic material between viruses that causes dramatic changes in the expression of antigens and is usually followed by serious outbreaks of infectious disease.

Antigen-presenting cells (APCs) process and display small peptides on the surface of cells in the context of MHC class I or class II proteins to present to receptors on T cells. Dendritic cells, B cells, and macrophages are principal APCs for T cells while follicular dendritic cells present antigen to B cells.

Antigens are molecules that react with antibodies, immunoglobulin surface receptors, or T-cell receptors.

Apoptosis, also known as *programmed cell death*, is an orderly progression of cellular death involving the degradation of genomic DNA with the eventual engulfment of cell debris. Antigen-activated lymphocytes undergo apoptosis after they have completed their effector functions, as a mechanism from keeping cells perpetually activated.

Arthus reaction occurs in the skin when antigen complexes with complement-fixing IgG antibodies, drawing phagocytic cells to the site with local inflammation.

Atopy is an allergic response mediated by IgE antibodies. IgE-allergen complexes bind to mast cells, triggering the release of histamine and other vasodilatory macromolecules.

Avidity is a collection of all the forces that control the binding of a receptor to its ligand or antibody to its antigen.

B7-1 and B7-2 are co-stimulatory molecules expressed on antigen-presenting cells and are critical in the activation of T cells.

Basophils are granulocytes that stain with basic dyes and mediate some aspects of inflammation.

Blood group antigens include the ABO and Rh antigens present on the

surface of red blood cells. They are the antigens used for blood bank typing.

Bone marrow is a primary lymphoid site from which all immune cells and red blood cells originate, including monocytes, granulocytes, lymphocytes, and platelets.

Bronchial-associated lymphoid tissues (BALT) are respiratory tract lymphoid tissues important in local respiratory infections and inhaled antigens.

Bursa of Fabricius is a lymphoid tissue of birds where B-lymphocyte development occurs. The first historical description of B-cell development occurred in this organ, although humans have no equivalent tissue.

Calcineurin is an important intracellular signaling molecule in the T cell and is the indirect target of immunosuppressive drugs such as cyclosporine and FK 506 bound to immunophilins.

Carrier proteins are foreign proteins used to bind nonimmunogenic small peptides or haptens to render them immunogenic.

CD (clusters of differentiation) refers to antigens typically located on the surface of cells and numerically notated. Approximately 125 CD antigens have been described to date, most of which are involved with various functions of the immune system.

CD3 complex is found next to the T-cell receptor and is critical in T-cell signaling through γ, Δ, ε, and ζ polypeptide chain components.

Cell-mediated immunity describes the adaptive immune response of T lymphocytes to a specific antigen.

Central lymphoid organs, sometimes termed primary lymphoid organs, include the bone marrow and thymus for B- and T-lymphocyte development, respectively.

Chédiak-Higashi syndrome is an immune deficiency disorder of phagocytic cells in which lysosomes are defective and do not kill intracellular bacteria.

Chemokines are small proteins within the cytokine family important in the attraction and activation of phagocytic cells and lymphocytes.

Chronic granulomatous disease is an immune deficiency syndrome of phagocytic cells due to a defect in the NADPH oxidase system. This results in an inability to make superoxide molecules, which kill intracellular bacteria.

Classical complement pathway is activated by antibody-antigen immune complexes and begins with C1, C4, followed by C2, as the earliest reactions in complement activation. Individual complement products have unique biologic properties such as anaphylatoxins (mediators of inflammation), opsonization of pathogens, chemoattraction, and the lysis of cells by the terminal complement proteins C7, C8, and C9.

Clonal expansion occurs when lymphocytes multiply after antigenic stimulation. This mechanism allows for specific immune responses to a pathogen to expand and clear the infection. Memory immune cells also develop in this adaptive immune response.

Combinatorial diversity describes the joining of various gene segments (such as V, D, and J genes) to generate a diverse immune response.

Common variable immunodeficiency is a syndrome resulting in defective antibody production of unknown etiology, although defects in genes mapping within the MHC complex are associated.

Complement is a collection of plasma proteins that may directly bind to pathogens or become activated by antibody bound to its antigen and initiate the complement cascade. The end product of complement activation is lysis of the pathogen or infected target cell. Individual products of complement activation have several biologic properties including anaphylatoxins, opsonization, and chemoattraction.

Complement receptors are found on the surfaces of phagocytic cells and allow for the uptake and removal of complement-bound pathogens.

Complementarity determining regions (CDRs) are the variable sites located within immunoglobulin or T-cell receptors that control antigen-binding specificity.

Contact hypersensitivity is a T cell–mediated delayed hypersensitivity reaction. The skin response to poison ivy is one example.

Convertase is a biochemical reaction that converts individual complement proteins in the complement cascade into an active form.

Coombs' test is an Rh incompatibility test for determining whether red blood cells are coated with antibody, by an agglutination technique using anti-immunoglobulin antibody.

Corticosteroids are natural hormones produced in the adrenal cortex. Purified and used as a drug, they are immunosuppressive in killing lymphocytes (primarily thymocytes) and in antiinflammatory responses.

Co-stimulatory molecules are cell surface proteins that are usually required for the full activation of T cells. They include B7-1 and B7-2 located on the surfaces of antigen-presenting cells, which bind to T-cell molecules, CD28 and CTLA-4. CD40 ligand on T cells binding to CD40 on B cells is another co-stimulatory ligand-receptor pair.

C-Reactive protein is an acute-phase protein that binds to the surface of bacteria and facilitates their uptake and destruction by phagocytic cells.

Cyclophosphamide is an immunosuppressive alkylating drug that cross-links DNA and kills rapidly dividing cells such as lymphocytes.

Cyclosporine is an immunosuppressive drug that inactivates the calcineurin signaling pathway in T lymphocytes.

Cytokines, also known as *lymphokines* or *interleukins*, are soluble proteins secreted by immune cells that have a variety of biologic functions.

Cytotoxic T cells are CD8-bearing T lymphocytes that bind to antigen in the context of MHC class I molecules. These cells have the ability to kill cells infected with intracellular pathogens by their secretion of perforins.

Delayed-type hypersensitivity is inflammation mediated by CD4 T cells arising hours to days after their interaction with antigen. An example of this immune response is in *Mycobacterium tuberculosis* skin testing. This immune response is in contrast to immediate hypersensitivity, which is mediated by antibody and observed minutes after exposure to antigen.

Dendritic cells are antigen-presenting cells with a characteristic elongated and branching morphology. They are important in presenting antigen to T cells.

DiGeorge syndrome is a T-cell immunodeficiency disease due to defective thymic epithelium development and an absence of parathyroid glands.

DNA-dependent protein kinase regulates the V-D-J gene recombination. Defects in this enzyme lead to severe combined immunodeficiency disease (SCID).

Efferent lymphatic vessels are routes from which lymphocytes exit a lymph node leading to the thoracic duct and back into the peripheral circulation.

Endotoxin, or *lipopolysaccharide (LPS)*, is a gram-negative bacterial cell wall product that causes the activation of B lymphocytes and the release of inflammatory cytokines. Sepsis may cause the systemic release of LPS, leading to endotoxic shock.

Enzyme-linked immunosorbent assay (ELISA) is a laboratory test able to detect small amounts of specific antibodies or antigens. The presence of antigen or antibody is detected by a secondary antibody-enzyme complex, leading to a colored product in the experimental well. It is a common laboratory procedure in the diagnoses of infectious diseases and autoimmune diseases.

Eosinophils are leukocytes in the granulocyte family important in the defense against parasites.

Epstein-Barr virus (EBV) is a member of the herpesvirus family that

binds to complement receptor 2 (CR2) on human B cells. It is the agent of infectious mononucleosis.

Erythroblastosis fetalis is a syndrome in which maternally derived antibodies to Rh red blood cell antigen cross the placenta and enter the fetus, producing severe hemolytic disease by the gradual depletion of red blood cells. The fetus is left with primarily immature red blood cells in its blood.

Fas is a cell surface protein on many lymphocytes that signals programmed cell death (apoptosis) when bound by its ligand, the Fas ligand. It is a member of the tumor necrosis factor receptor family.

Fc receptors are composed of 3 classes, R1, R2, and R3, all binding the Fc region of aggregated IgG. Fc are found on macrophages, B cells, follicular dendritic cells, and natural killer cells.

FK 506 is an immunosuppressive drug commonly used in organ transplantation to prevent graft rejection, by inhibiting T-cell signal transduction from the T-cell receptor.

Germinal centers are areas within lymphoid tissues where B-cell maturation and replication take place. Germinal centers are rich in follicular dendritic cells, which maintain and present immune complexes to the B cell for activation in the development of memory.

Goodpasture's syndrome is a lethal autoimmune disease in which autoantibodies to type IV collagen and basement membrane create severe vasculitis.

Graft-versus-host disease occurs when lymphocytes from engrafted tissue, usually allogeneic bone marrow, attacks tissues in an immunosuppressed host.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a growth factor for dendritic cells, monocytes, tissue macrophages, and granulocytes.

Granuloma is a nodule where chronic inflammation occurs from persistent infections. Granulomas consist of a dense population of T cells and macrophages.

Graves' disease is an autoimmune hyperthyroid disease caused by autoantibodies to thyroid-stimulating hormone.

Gut-associated lymphoid tissues (GALT) are sites of lymphocyte development and activation found in the GI tract. These tissues process antigens from consumed food and normal intestinal bacteria for exposure to lymphocytes.

Haplotype is a term used to describe a set of MHC genes in an individual or species. Linkage disequilibrium is when closely linked MHC genes are co-expressed in successive generations and do not segregate by normal statistics.

Haptens are small molecules that cannot elicit humoral immunity unless bound to a larger protein called a carrier.

Hashimoto's thyroiditis is inflammation of the thyroid gland caused by autoantibodies to thyroid-specific antigens, leading to an influx of macrophages and natural killer cells to the thyroid tissue.

Heavy chain of immunoglobulin molecules confer specific biologic properties to the molecule, including isotype, ability to bind Fc receptor, and distinctive allotypes. Immunoglobulin molecules consist of 2 heavy chains, each of 50 to 70 kd.

Human immunodeficiency virus (HIV) is the viral infectious agent responsible for acquired immune deficiency syndrome (AIDS).

Immunoblotting is a diagnostic laboratory technique in which proteins are separated by electrophoresis and transferred to paper. Specific proteins are then identified by the presence of labeled antibodies. The technique is useful in defining antibody responses to proteins of various pathogens (including HIV).

Immunodiffusion is a historically important laboratory technique in which antibodies and antigens diffuse through gelatin, forming a visible immunoprecipitation line.

Immunofluorescence is a laboratory technique in which serum antibodies are examined for binding to intracellular antigens or to pathogens fixed to glass slides. Specific antibody binding is detected by a fluorescent second antibody.

Immunoglobulins, also known as *antibodies*, are soluble, antigen-binding proteins secreted by activated B lymphocytes. Immunoglobulin (Ig) has 5 isotypes known as IgG, IgA, IgM, IgE, and IgD.

Immunologic memory describes the rapid immune recall response when antigen is presented to the immune system more than once. Memory is the basis for effective vaccine design, since it is a specific and long-lived immune response.

Immunophilins are intracellular proteins that are targets of the immunosuppressive drugs FK 506 and cyclosporine.

Inflammation describes the influx of plasma fluids and leukocytes into sites of injury or infection.

Innate immunity describes the variety of non-specific defense mechanisms against pathogens. It is the first line of immune defense and does not change over time in an individual. Innate immunity is followed in time by B- and T-cell adaptive immune responses to the pathogen.

Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease in which the β cells of the pancreas are destroyed, leaving deficient insulin production.

Integrins are adhesion molecules on the surface of leukocytes that aid in their migration into tissues. The ligands of integrins are the ICAM molecules on the surfaces of endothelial cells.

Intercellular adhesion molecules (ICMs) are cell-surface proteins that bind LFA-1. ICAM-LFA-1 binding mediates interactions between leukocytes and either antigen-presenting cells or endothelial cells.

Interleukin is a general term for cytokines synthesized and secreted by leukocytes.

Isotype describes the various subclasses of immunoglobulin molecules including IgM, IgG, IgD, IgA, and IgE. Isotype is encoded by the heavy-chain constant region, which determines in part the biologic activities of the antibody.

Isotype switching is when site-specific recombination changes IgM antibody into IgG. Switching does not alter antigen specificity but can change the biologic functions of the antibody.

Langerhans' cells are dendritic cells located in the skin that are able to migrate with antigen to local lymph nodes.

Lentiviruses are a collection of retroviruses to which the HIV belongs.

Leukocyte describes the collection of white blood cells including lymphocytes, monocytes, and polymorphonuclear leukocytes.

Leukocyte adhesion deficiency is a disease of defective integrin production not allowing leukocytes to migrate to sites of infection.

LFA-1 (lymphocyte function–associated antigen-1) is a T-cell surface integrin molecule that binds to ICAM on the surface of endothelial cells and antigen-presenting cells, allowing for T-cell migration and adhesion at sites of infection.

Light chains of immunoglobulin are 20 to 25 kd polypeptides that are non covalently linked to the heavy chain polypeptides. Light chains are found as two types, kappa and lambda, and contribute to antigen-binding specificity of immunoglobulin molecules.

L-selectin is a lymphocyte surface adhesion molecule binding to glyCAM-1 present on high endothelial venules (HEVs). The interactions of adhesion molecules help the migration of naive lymphocytes into lymphoid tissues.

Lymphatics are a system of channels that drain fluid from peripheral tissues through the thoracic duct back into the blood.

Lymph nodes are known as secondary lymphoid tissues to which local antigens drain and initiate specific adaptive B- and T-cell immune responses.

Lymphotoxin (tumor necrosis factor-β, TNF-β) is a cytokine synthesized by CD4 T cells directly cytotoxic to some tumor cells and other target infected cells.

Macrophages are bone marrow–derived phagocytic cells that play a

role in innate immunity via phagocytosis of pathogens and function to present antigen to other cells such as T cells.

Major histocompatibility complex (MHC) is the collection of immune reactive genes that encode MHC class I and class II molecules or class III proteins (complement proteins). The genes are found on human chromosome 6 (termed HLA) or on mouse chromosome 17 (termed H2).

Mast cells are important cells in allergic reactions because their Fc receptors bind IgE antibody, causing degranulation and the release of vasoactive amines. Mast cells are found throughout the body in connective tissues and in skin.

Membrane attack complex describes the terminal complement components C7, C8, and C9, which create pores and eventual lysis of a target cell.

Minor histocompatibility antigens, also known as *minor H antigens*, are MHC-bound peptides that are important in T cell–mediated graft rejection.

Mixed lymphocyte reactions define the T-cell proliferation that occurs when cells from 2 different individuals with different HLA antigens are mixed together. These reactions are performed for histocompatibility testing in allograft transplantation.

Monoclonal antibodies are antibodies synthesized by a single clone of B lymphocytes with single specificity. The secreting cells are generated in the laboratory by the fusion of a B cell with an immortal myeloma tumor cell.

Monocytes are bone marrow–derived cells that develop into macrophages.

Mucosal-associated lymphoid tissue (MALT) consists of all epithelial tissues and lamina propria containing lymphoid cells near mucosal surfaces. These tissues include those found in the gut (GALT) and in the bronchial tract (BALT).

Multiple myeloma is a proliferating plasma cell tumor (B cell) that secretes a monoclonal immunoglobulin termed myeloma protein.

Multiple sclerosis is a T cell–mediated autoimmune neurological disease characterized by demyelination in the central nervous system.

Myasthenia gravis is an autoimmune disease mediated by autoantibodies specific to the acetylcholine receptor within muscle cells. The inhibition of neuromuscular signaling causes progressive muscle weakness leading to death.

Natural killer cells (NK cells) directly kill some tumor cells and some virus infected cells as well as participate in antibody dependent cell mediated cytotoxicity (ADCC). NK cells are not derived from either B or T lymphocytes but have a distinctive granular shape.

Neutrophils are major phagocytic cells for killing extracellular pathogens and are characterized by a multilobed nucleus.

Opsonization is the process by which phagocytic cells such as neutrophils or macrophages engulf and digest a pathogen. Antibody or complement may bind to the pathogen, followed by attachment to specific receptors (Fc or C′ receptors) on phagocytes.

Paroxysmal nocturnal hemoglobinuria (PNH) is the hemolysis of red blood cells due to a defect in production of complement regulatory proteins.

Passive immunization is the act of supplying immunoglobulins from an immune donor into a naive recipient.

Periarteriolar lymphoid sheath (PALS) is a site of T-cell development within the white pulp of the spleen.

Peripheral lymphoid organs, also known as *secondary lymphoid organs*, include the spleen, lymph nodes, and mucosal-associated lymphoid tissue where specific B- and T-cell immune responses develop.

Peripheral tolerance occurs outside the central lymphoid organs in developing lymphocyte nonresponsiveness to various self-antigens.

Peyer's patches are lymphoid organelles containing B cells and some T cells in the small intestine. Peyer's patches are sites of lymphocyte exposure and development and development to antigenic stimulation and development.

Phagocytosis is the engulfment and internalization of pathogens or other particles by macrophages or neutrophils. Intracellular lysosomal enzymes degrade the pathogen inside of a phagosome.

Plasma cells are antibody-secreting B lymphocytes. Plasma cells are the final stage of development of B lymphocytes that have been stimulated by antigen.

Polymorphism describes nucleotide differences that exist in a particular gene between individuals, resulting in amino acid sequence changes in the protein product. Genes of the MHC are polymorphic in that many types have been described at a single locus (i.e., HLA A1, HLA A2, etc.).

Polymorphonuclear leukocytes, also known as *granulocytes*, are leukocytes with lobed nuclei and cytoplasmic granules. Three polymorphonuclear leukocyte types are basophils, neutrophils, and eosinophils.

Prednisone is an antiinflammatory and immunosuppressive drug used to treat inflammatory responses in autoimmune disease and in acute graft rejection.

Programmed cell death (see *Apoptosis*).

Properdin (factor P) acts with complement protein C3bBb in the activation of the alternative complement pathway.

Proteasome is the intracellular site where antigens get degraded for presentation by MHC class I molecules.

Purine nucleotide phosphorylase deficiency is one cause of severe combined immunodeficiency disease (SCID). The defect causes an accumulation of intracellular purine nucleosides, toxic to developing lymphocytes.

Reagins (reaginic antibodies) are historically defined as atopic IgE antibodies responsible for immediate hypersensitivity (allergy).

Receptor editing is the process by which B-cell surface immunoglobulin changes specificity during development to eliminate self-reactivity.

Recombination signal sequences are portions of DNA on either side of variable region gene segments that control gene rearrangements. The signal sequence is a 7- or 9-nucleotide segment separated by 12 or 23 nucleotides. These sequences keep nonproductive rearrangements (V-V, D-D, etc.) from occurring.

Reverse transcriptase is a retroviral enzyme that converts RNA into a copy of DNA for integration into the host cell genome. A synthetic form of reverse transcriptase is utilized for DNA cloning in molecular biology.

Rh blood group antigens are targets of nonagglutinating anti-Rh antibodies. Rh antigen is the target of antibodies in erythroblastosis fetalis and is detected by the Coombs' test.

Serum sickness is caused by the formation of immune complexes, typically when foreign gammaglobulins are administered to an individual eliciting an anti-immunoglobulin response in the host. Symptoms include kidney disease, fever, and arthralgias.

Severe combined immune deficiency disease (SCID) is characterized by an absence of both B- and T-cell immune responses. Several defects in cellular metabolism can elicit SCID.

Somatic mutation is the process where antibodies undergo nucleotide changes that typically increase affinity and specificity to their antigen after secondary exposure to antigen.

Staphylococcal enterotoxins are the etiologic agent of food poisoning and also nonspecifically stimulate T cells by direct binding to the T-cell receptor. Staphylococcal enterotoxins are also known as *superantigens*.

Suppressor T cells are CD8 T cells that downmodulate other lymphocyte subsets by mechanisms that are as yet poorly defined.

Systemic anaphylaxis is an immediate hypersensitivity response where systemic release of vasoactive amines causes the dilation of

blood vessels and the subsequent accumulation of fluids in the tissue, often leading to death.

TAP proteins are two intracellular proteins that transport digested peptides into the endoplasmic reticulum for assembly with MHC class I molecules.

Thoracic duct runs along the aorta through the abdomen and functions to return lymphatic fluid and lymphocytes back into the peripheral blood via the left subclavian vein.

Thymus is a primary lymphoid organ where T-cell development occurs from pluripotent bone marrow stem cells.

Tolerance is the term used to describe the mechanisms that instruct lymphocytes to ignore self-antigens. Tolerance mechanisms can occur in central organs such as the bone marrow and thymus where self-antigens combined with MHC proteins are presented to lymphocytes. *Peripheral tolerance* to various tissue antigens can occur outside primary lymphoid organs.

Tuberculin test is performed by the injection of *Mycobacterium tuberculosis* extract under the skin to elicit T cell–mediated delayed-type hypersensitivity in *M. tuberculosis*–infected individuals.

Type I hypersensitivity reactions are caused by IgE-antigen complex triggering of mast cells.

Type II hypersensitivity is due to IgG class antibodies directed at cell surface antigens.

Type III hypersensitivity is initiated by antigen-antibody complexes.

Type IV hypersensitivity is mediated by T lymphocytes.

Variable region (V region) of immunoglobulin is formed by the association of V, D, and J gene segments during antibody development. It represents the most variable amino acid sequences within the immunoglobulin structure.

Wiskott-Aldrich syndrome is a congenital immunodeficiency disorder causing defects in antibody production to bacterial polysaccharide antigens. Individuals have recurrent pyogenic bacterial infection.

X-linked agammaglobulinemia is caused by a genetic defect in the synthesis of protein tyrosine kinase BTK. B-cell development is inhibited and no subsequent antibodies are synthesized.

X-linked hyper IgM syndrome is a congenital defect in expression of the CD40 ligand, a surface protein found on activated T cells. The defect causes an absence of IgG, IgE, or IgA antibody production, although serum IgM levels are elevated.

X-linked severe combined immunodeficiency (X-linked SCID) is caused by a defect in cytokine receptors, leading to a failure of T-cell development. As a consequence, T-cell help for antibody production is absent.

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